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(54) Title: Wnt RECEPTOR COMPOSITIONS AND METHODS

(57) Abstract

Wnt receptor compositions and methods of use are disclosed. In particular, methods using Wnt receptors, such as Dfz2, in screens for compounds which modulate the binding of a Wnt polypeptide to a Wnt receptor.

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WNT RECEPTOR COMPOSITIONS AND METHODS

FIELD OF THE INVENTION

The present invention relates to screening methods employing Wnt receptors.

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BACKGROUND OF THE INVENTION

Wnt genes encode secreted proteins involved in cell-to-cell signaling. *Wnt* genes play important growth controlling roles, in particular in the mammary gland, and act as oncogenes in mouse mammary tumors. Little is known about the mechanism of action of *Wnt* products, in part because Wnt receptors have until now remained unidentified.

SUMMARY OF THE INVENTION

In one aspect, the present invention includes an isolated nucleic acid molecule encoding a Wnt receptor polypeptide. In a general embodiment, the Wnt receptor polypeptide has an amino acid sequence that is greater than about 90% identical to the

amino acid sequence of a Wnt receptor selected from the group consisting of Dfz1, Dfz2, Rfz1, Rfz2, Hfz3, Hfz4, Hfz5, Mfz3, Mfz4, Mfz5, Mfz6, Mfz7, Mfz8, and Cfz1. In a related embodiment, the Wnt receptor has an amino acid sequence that is more than about 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16. In another related embodiment, the Wnt receptor polypeptide has an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16.

Examples of nucleic acid molecules encoding Wnt receptor polypeptides are provided herein as SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13 and SEQ ID NO:15. Preferred embodiments are human Wnt polynucleotides. An exemplary human Wnt polynucleotide has the sequence presented as SEQ ID NO:9.

The invention further includes fragments of polynucleotides encoding full-length WntR, where the fragments are of sufficient length to hybridize selectively with a Wnt polynucleotide sequence or complement thereof, such as a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13 and SEQ ID NO:15. Such fragments are at least 15, preferably at least about 18, 21 or 24, nucleotides in length.

In another aspect, the invention includes an isolated Wnt receptor polypeptide. In a general embodiment, the polypeptide has an amino acid sequence that is more than about 90% identical to the amino acid sequence of a Wnt receptor selected from the group consisting of Dfz1, Dfz2, Rfz1, Rfz2, Hfz3, Hfz4, Hfz5, Mfz3, Mfz4, Mfz5, Mfz6, Mfz7, Mfz8, and Cfz1. In a related embodiment, the polypeptide has an amino acid sequence that is more than about 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16. In another related embodiment, the polypeptide sequence is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16.

Preferred embodiments are human Wnt polypeptides. An exemplary human Wnt polypeptide has the sequence presented as SEQ ID NO:10.

The invention further includes peptide fragments derived from a full-length WntR polypeptide, where the fragments contain a region of at least seven, preferably at least ten, consecutive amino acids, and where the region has at least about an 80% identity with the residues of a corresponding region of a polypeptide having a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16.

Also included in the invention are antibodies, both monoclonal and polyclonal, specifically-immunoreactive with Wnt receptor polypeptides. Such antibodies may be produced using standard methods (Harlow).

The invention also includes a method of identifying a compound capable of affecting binding of a Wnt polypeptide to a Wnt receptor polypeptide. The method includes (i) contacting such a Wnt receptor polypeptide with a selected Wnt polypeptide, in the presence and absence of a test compound, (ii) measuring the effect of the test compound on the extent of binding between the Wnt polypeptide and the Wnt receptor polypeptide, and (iii) identifying said compound as effective if its measured effect on the extent of binding is above a threshold level. In a general embodiment, the method includes an additional step (iv) comprising preparing a pharmaceutical preparation of a compound identified as effective to alter binding of a Wnt polypeptide to a WntR polypeptide.

In one embodiment, the threshold is a 2-fold or greater inhibition of binding. In another embodiment, the threshold is a 2-fold or greater potentiation of binding. Examples of suitable Wnt polypeptides include *wingless* (Wg); examples of suitable Wnt receptor polypeptides include Dfz2 (e.g., SEQ ID NO:2).

The test compound may be effective to inhibit binding between the Wnt polypeptide and the Wnt receptor or to displace the Wnt polypeptide from the Wnt receptor polypeptide. In one embodiment, the Wnt receptor polypeptide is expressed on the surface of a cell (e.g., Drosophila Sneider 2 (S2) cell) transformed with an expression vector encoding said receptor (e.g., Dfz2).

In another embodiment, the Wnt receptor polypeptide is an N-terminal portion of a full-length Wnt receptor polypeptide, the N-terminal portion including the cysteine-rich amino-terminal domain. In one embodiment, the N-terminal portion is part of a fusion with, e.g., the constant domain of human IgG.

These and other objects and features of the invention will become more fully apparent when the following detailed description is read in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows a sequence comparison of Dfz1 and Dfz2.

Figure 2 shows hydropathy profiles of mammalian and nematode frizzled homologues.

Figure 3 shows a computer-generated image of the expression of DFz2 during Drosophila development evaluated by Northern blot.

Figure 4 is a computer-generated image showing that transfection of DFz2 into S2 cells confers a response to Wg protein.

Figure 5 is a computer-generated image made using confocal immunomicroscopy showing binding of Wg protein to Dfz-2 transfected cells.

Figure 6 is a computer-generated image showing the binding of metabolically labeled Wg protein to a Dfz-2/Ig fusion protein.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

A polynucleotide sequence or fragment is "derived from" another polynucleotide sequence or fragment when it contains the same sequence of nucleotides as are present in the sequence or fragment from which it is derived. For example, a bacterial plasmid contains an insert "derived from" a selected human gene if the sequence of the polynucleotides in the insert is the same as the sequence of the polynucleotides in the selected human gene.

Similarly, a polypeptide sequence or fragment is "derived from" another polypeptide sequence or fragment when it contains the same sequence of amino acids as are present in the sequence or fragment from which it is derived. A polypeptide "derived from" a nucleic acid is a polypeptide encoded by that nucleic acid. For example, a Wnt receptor polypeptide derived from the human genome (also termed "human Wnt receptor polypeptide" or "hWntR") is a polypeptide encoded by an mRNA (or corresponding cDNA) transcribed from a human Wnt receptor gene.

Percent (%) identity, with respect to two amino acid sequences, refers to the % of residues that are identical in the two sequences when the sequences are optimally aligned

and no penalty is assigned to "gaps". In other words, if a gap needs to be inserted into a first sequence to optimally align it with a second sequence, the % identity is calculated using only the residues that are paired with a corresponding amino acid residue (i.e., the calculation does not consider residues in the second sequences that are in the "gap" of the first sequence). Optimal alignment is defined as the alignment giving the highest % identity score. Such alignments can be preformed as described herein using the "GENEWORKS" program. Alternatively, alignments may be performed using the local alignment program LALIGN with a ktup of 1, default parameters and the default PAM. The LALIGN program is found in the FASTA version 1.7 suite of sequence comparison programs (Pearson and Lipman, 1988; Pearson, 1990; program available from William R. Pearson, Department of Biological Chemistry, Box 440, Jordan Hall, Charlottesville, VA).

A full-length Wnt receptor (WntR) polypeptide is defined herein as a polypeptide that is a member of the frizzled protein family, encodes a full-length protein, and has at least about a 90% identity with one or more of the following sequences: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14 and SEQ ID NO:16.

II. Overview of the Invention

The present invention is based on the discovery of a set of novel members of the vertebrate frizzled family of polarity genes, and on the recognition that the frizzled family of polarity genes encodes the receptors for the Wnt family of proteins. The invention is further enhanced by the recognition that the full-length sequence of each member of the frizzled protein family generally shares a substantially greater degree of homology with the full-length sequences of corresponding frizzled proteins in other species (typically about 80% to >95%) than it does with the full-length sequences of other members of the frizzled protein family in the same species (typically about 30% to 60%). Different members of the frizzled family, however, do contain regions within the coding sequences that have high degrees of homology (up tp 90% or more) with one another. This feature, combined with similar sizes and hydrophobicity profiles, facilitates the identification of novel members of the frizzled gene family.

Discoveries described herein enable a number of uses and application of the present invention. These uses and applications are exemplified and discussed in detail below.

III. Identification of Dfz2 as the Wg Receptor

Experiments performed in support of the present invention and described in Examples 1-6, below, indicate that *Drosophila frizzled* gene 2 (Dfz2) is a receptor for *wingless* (Wg). Example 1 details the cloning of Dfz2, the sequence of which is illustrated in Figure 1. Hydrophobicity profiles of additional frizzled family members isolated as part of the present invention are shown in Figure 2. Their sequences are presented in the Sequence Listing. Example 2 describes *in situ* hybridization experiments to determine the pattern of Dfz2 expression. Example 3 describes Northern analyses (Fig. 3) showing that Dfz2 is expressed throughout development.

In Example 4, below, *Drosophila* Sneider 2 (S2) cells were transformed with a Dfz2 expression vector and the effects of the Dfz2 ligand, Wg, were assessed by measuring the levels of *armadillo* (Arm) protein in response to Wg application (Peifer, *et al.*, 1994; Riggleman, *et al.*, 1990; Van Leeuwen, *et al.*, 1994). The results, shown in Figure 4, demonstrate that all four Dfz2-transfected S2 cell lines tested showed increased armadillo signal in response to Wg, whereas no such effect was observed with untransfected S2 cells. These results demonstrate that Dfz2 acts as a signal transducing molecule for Wg, consistent with it being a receptor for Wg.

Further support is provided by immunohistochemical analyses described in Example 5. These experiments were designed to address whether Wg was capable of binding to the Dfz2-transfected cells. Dfz2-transfected and nontransfected cells were exposed to medium containing Wg protein, washed, stained with an anti-Wg antiserum and a labelled secondary antibody, and imaged using a confocal microscope. Exemplary images, shown in Figs 5A-5F, demonstrate that approximately 80% of Dfz2-transfected S2 cells exposed to Wg protein stained brightly (Fig. 5D) whereas Dfz2-transfected cells in the absence of Wg protein (Fig. 5A) as well as non transfected S2 cells (Fig. 5B) did not. The ability of Wg to bind was also tested in human 293 cells, which are heterologous to the Dfz2 protein. As shown in Fig. 5F, about 10-20% of the transfected cells remained positive, similar to the transfection efficiency of 293 cells. Since 293 cells are of human origin, these results indicate that Wg binds to Dfz2 itself, rather than to a molecule whose expression is induced by Dfz2.

The binding of Wg protein to Dfz2 was further confirmed using a fusion protein containing the cysteine-rich amino-terminal domain of Dfz2, linked to the constant domain of human IgG, as described in Example 6. The fusion protein or IgG control was added to conditioned medium from normal S2 cells, or S2 cells producing Wg (HS-wg/S2), which had been metabolically-labeled with [^{35}S] cysteine and methionine.

The fusion proteins and possible complexes were then isolated and analyzed by gel electrophoresis and fluorography (Fig. 6). Two bands of approximately 52 kd (the size of Wg) were detected in the lane with the Dfz2-Ig fusion added to the medium of HS-wg/S2 cells.

The above results taken together, particularly the observations that (i) Wg binds to DFz2, and (ii) the binding leads to a biological response, strongly support the role of Dfz2 as the receptor for the Wg protein.

IV. Novel Frizzled Family Members Identified in Vertebrates

Experiments performed in support of the present invention have further resulted in the identification of at least six novel frizzled family members in human and mouse. This brings the total number of frizzled-like sequences identified in mammalian genomes to 8, since two (Rfz1 and Rfz2) were previously cloned from rat (Chan, *et al.*, 1992). The six novel genes include Mfz3, Mfz4, Mfz6, Mfz7, and Mfz8, as well as human sequences Hfz3, Hfz5 and Hfz7. A sequence 95% identical over 143 amino acids to Hfz5 was PCR-amplified (Mullis, 1987; Mullis, *et al.*, 1987) from mouse genomic DNA using Hfz5-specific primers, suggesting that an Mfz5 gene exists as well. The DNA and translated amino acid sequences of these 6 family members are provided in the Sequence Listing, along with the sequence of a novel family member isolated from C. elegans (Cfz1). The hydrophobicity profiles of these sequences are presented in Figure 2. These profiles, along with the sequences of regions that are conserved among different frizzled family members, are used in determining whether a polypeptide sequence is a member of the frizzled gene family. According to the present invention, member of this family are considered to be Wnt receptors.

Using the guidance herein, one of skill in the art can isolate additional members of the frizzled gene family. In particular, probes homologous to regions conserved among the various family members can be designed and used to probe cDNA or genomic DNA libraries. Alternatively or in addition, PCR primers corresponding to such conserved regions may be designed and used to isolate additional sequences from any suitable source of DNA, including libraries and reverse transcription (RT) -generated cDNA samples.

V. Wnt Genes and Proteins

Wg in Drosophila is part of larger gene family (Eisenberg, *et al.*, 1992; Graba, *et al.*, 1995; Russell, *et al.*, 1992) of Wnt genes. At least 3 homologous genes have been

identified in *Drosophila*, and over 10 Wnt genes have been identified in most vertebrates (Nusse and Varmus, 1992). According to the present invention, the products of these genes are the ligands for receptors encoded by the large family of fz-like genes in vertebrates. Determination of which Wnt gene products are specific to which Wnt receptor may be performed by one of skill in the art following the teachings of the present specification.

All members of the *Wnt* family encode secreted proteins that act as cell-cell signaling molecules. *Wnt* genes play an important role in the control of cell growth, particularly in the mammary gland, and can act as oncogenes in mouse mammary tumors. The proteins contain a signal sequence, one or several N-linked glycosylation sites and many cysteine residues. The product of the mouse *Wnt*-1 gene has been studied most extensively. If *Wnt*-1 is overexpressed in various cell lines, the protein enters the secretory pathway. The protein can be detected in protease resistant structures, presumably secretory vesicles, and contains carbohydrate structures at several N-linked glycosylation sites. It is thus generally assumed that the *Wnt*-1 protein is secreted from cells, although extracellular forms of the protein have been difficult to detect. In addition, most of the intracellular *Wnt*-1 protein made in transfected cells is incompletely glycosylated (it remains sensitive to endoglycosidase H) and has probably not traversed the Golgi apparatus. Moreover, much of the *Wnt*-1 protein becomes associated with the resident ER protein BiP, indicating that it is incorrectly folded.

In spite of these difficulties, it has been shown that *Wnt*-1 overproduction leads to secretion of modest amounts of extracellular protein. The secreted forms have undergone more extensive glycosylations, and may bind to the cell surface or to the extracellular matrix.

VI. Role of Wnt in Cancer

Members of the *Wnt* gene family are important regulators of mammary cell growth. Indeed, *Wnt* genes owe their discovery to their role as oncogenes in mouse mammary cancer: previous experiments which examined the sequence around integration sites for Mouse Mammary Tumor Virus (MMTV) DNA showed that many tumors had sustained proviral insertions near the *Wnt*-1 gene, the first member of this gene family. A biological assay for *Wnt*-1 was subsequently established using gene transfer experiments. This assay was used to show that certain mammary gland-derived cell lines can be morphologically transformed by *Wnt*-1. Direct evidence that *Wnt*-1 expression gives a strong growth stimulus to mammary cells came from transgenic mice carrying *Wnt*-1 linked to the MMTV

promoter, which developed mammary hyperplasia and tumors. By infecting primary mammary cells with retroviruses expressing *Wnt*-1 and re-implantation of the infected cells, similar hyperplasia of the mammary gland were obtained. Additional experiments led to the identification of a *Wnt*-1 related oncogene activated by MMTV insertion, called *Wnt*-3.

The growth stimulus generated by the expression of *Wnt*-1 in the mammary gland implies that mammary cells are equipped with a Wnt receptor that becomes activated by the *Wnt*-1 protein, as well as the other signaling components. While neither *Wnt*-1 nor *Wnt*-3 are expressed in the normal mammary gland, at least 5 other *Wnt* genes are expressed during specific stages of mammary gland development, including during the rapid expansion of the pre-lactating gland or when the gland regresses.

The oncogenic action of *Wnt*-1 and *Wnt*-3 is best explained by their acting as ligands for Wnt receptors meant for other *Wnt* genes, and activating these receptors inappropriately. Alternatively, *Wnt*-1 and *Wnt*-3 may not activate these receptors but may interfere with a ligand-receptor interaction normally leading to regression of the gland.

The strong growth stimulus by oncogenic *Wnt* genes and the dynamic expression patterns of other *Wnt* genes in the mammary gland provide evidence that *Wnt* genes are important regulators of mammary gland growth. It is also possible that *WNT* genes other than *WNT*-1 and *WNT*-3 are involved in human breast cancer. In analogy with the mouse, it is likely that some of these are expressed during the normal cycles of growth of the mammary gland. In contrast to silent genes, genes that are expressed are candidates to become amplified, since the ensuing overexpression of those genes can give a selective advantage to cells even during the first rounds of amplification.

By way of illustration, a survey of mouse mammary tumors identified one tumor where the mouse *Wnt*-2 gene was amplified and overexpressed whereas *Wnt*-2 had a low level of expression in the normal gland. Further, there was no evidence for insertion of MMTV near *Wnt*-2 in that tumor. This finding shows that *Wnt* genes are not necessarily activated only by MMTV, a relevant factor for human breast cancer since that disease has no viral etiology but is often characterized by gene amplification.

VII. Screening Methods

In view of the role of Wnt in cancer and other processes involving growth, development and proliferation (both normal and abnormal), it would be desirable to identify modulators of Wnt activity that affect the interactions of specific Wnt proteins with their receptors. Such modulators may, for example, inhibit the binding of Wnt to its receptor

(*e.g.*, by competitive or noncompetitive inhibition), or they may potentiate or stabilize the binding. The recognition that members of the frizzled family of proteins can act as receptors for the Wnt family of proteins enables a number of screening approaches to the isolation of such modulatory compounds that have heretofore not been possible.

Examples of such screening approaches include protein-protein binding assays in which the level of binding of Wnt to its receptor, or a biological consequence of such binding, is measured. The latter assay is exemplified in Example 4, where cells not normally expressing Wnt receptors are transformed with a Wnt receptor (in this case, Dfz2), and the effects of Wnt (in this case, Wg) on the cells are measured (in this case, by detecting levels of Arm). Such cells may be transformed with the Wnt receptor of choice (e.g., any of fz1, fz2, fz3, fz4, fz5, fz6, fz7 or fz8 receptors).

In Example 4, expression of Arm was detected using a Western blot method. Other methods may be employed which are more suitable for high throughput screening applications. For example, labelled anti-Arm antibodies may be used to directly visualize levels of Arm in multi-well format screen.

Alternatively, the assays may simply detect the degree of binding between Wnt ligands and Wnt receptors, and not the biological consequences of such binding. For example, cells expressing a selected Wnt receptor may be plated in the wells of a 96-well plate and contacted with a solution containing reporter-labeled Wnt (e.g., radiolabelled of fluorescently-tagged) in the presence and absence of a test compound (*i.e.*, a putative modulator of Wnt/receptor interactions). The effect of the test compound on the extent of binding between Wnt and Wnt receptor is measured, and the compound is identified as effective if its effect on the extent of binding is above a threshold level (*e.g.*, a several-fold difference in binding level between control and experimental samples) In one embodiment, the threshold is a 2-fold difference. In another embodiment, it is a 5-fold difference. In yet another it is a 10-fold or greater difference. The difference in binding in the presence and absence of an effective test compound is preferably statistically-significant, as determined by a standard statistical test.

It will be appreciated that the putative modulator compound can alternatively be added after the cells had been incubated with labelled Wnt. In a screen for inhibitors of binding, the system is assayed for a decrease in the signal reflecting bound labelled Wnt, or an increase in the signal reflecting labelled Wnt in solution.

Such a screen may also be employed to screen for potentiators of Wnt/receptor interactions. For example, test compounds may be added to the wells (either during or

after incubation with labelled Wnt), and the wells then contacted with unlabeled Wnt. Test compounds in wells where the unlabelled Wnt is less effective at displacing the bound labelled Wnt are selected for more detailed examination of ability to potentiate Wnt/receptor binding.

Assays such as described above may also be used to determine the relationship between different Wnt proteins and different receptors. For example, the ligand concentration dependence of binding may be used in measurement of the relative affinities of selected Wnt receptors with selected ligands, and ligands with a selected affinity for the receptor can be examined further using, *e.g.*, *in vitro* or *in vivo* assays. In this manner, one of skill in the art can identify which Wnt protein(s) is optimally paired with which receptor(s).

In cases where the Wnt ligand has been matched to a specific Wnt receptor (e.g., in the case of Wg and Dfz2), the receptor/ligand pair can be used in, e.g., screening applications. For example, the pair may be used in a binding assay to screen for compounds which are effective to modulate the binding of the specific ligand to its receptor. These methods enable the identification of compounds with two general types of activities: (i) those which act generally, *e.g.*, on a class of Wnt/Wnt receptor pairs, to disrupt or facilitate binding, and (ii) those which act selectively disrupt or facilitate the binding between a selected Wnt ligand and its receptor, but not between other Wnt ligands and their receptors.

Compounds identified by one of the screens described herein may be further evaluated for efficacy using an in vitro assay such as described above. Further, such compounds may be tested in *in vivo* models employing Wnt/Wnt receptor interactions. For example, the compounds may be tested in a mouse mammary tumor model for effectiveness at inhibiting growth of mammary tumors.

VIII. Compounds Suitable for Screening

A variety of different compounds may be screened using methods of the present invention. They include peptides, macromolecules, small molecules, chemical and/or biological mixtures, and fungal, bacterial, or algal extracts. Such compounds, or molecules, may be either biological, synthetic organic, or even inorganic compounds, and may be obtained from a number of sources, including pharmaceutical companies and specialty suppliers of libraries (*e.g.*, combinatorial libraries) of compounds.

In cases where an identified active compound is a peptide, the peptide may be utilized to design a peptoid mimetic and aid in the discovery of orally-active small molecule mimetics. Alternatively, the peptides themselves may be used as therapeutics.

Further, the structure of a bioactive polypeptide may be determined using, for example, NMR, and may be used to select the types of small molecules screened.

Methods of the present invention are well suited for screening libraries of compounds in multi-well plates (*e.g.*, 96-well plates), with a different test compound in each well. In particular, the methods may be employed with combinatorial libraries. A variety of combinatorial libraries of random-sequence oligonucleotides, polypeptides, or synthetic oligomers have been proposed (Kramer, *et al.*, 1993; Houghten, 1985, 1994; Houghten, *et al.*, 1986, 1991, 1992; Ohlmayer, *et al.*, 1993; Dooley, *et al.*, 1993a-1993b; Eichler, *et al.*, 1993; Pinilla, *et al.*, 1992, 1993; Ecker, *et al.*, 1993; and Barbas, *et al.*, 1992). A number of small-molecule libraries have also been developed (*e.g.*, Bunin, *et al.*, 1994; Bunin and Ellman, 1992; Virgilio and Ellman, 1994).

Combinatorial libraries of oligomers may be formed by a variety of solution-phase or solid-phase methods in which mixtures of different subunits are added stepwise to growing oligomers or parent compound, until a desired oligomer size is reached (typically hexapeptide or heptapeptide). A library of increasing complexity can be formed in this manner, for example, by pooling multiple choices of reagents with each additional subunit step (Houghten, *et al.*, 1991).

Alternatively, the library may be formed by solid-phase synthetic methods in which beads containing different-sequence oligomers that form the library are alternately mixed and separated, with one of a selected number of subunits being added to each group of separated beads at each step (Furka, *et al.*, 1991; Lam, *et al.*, 1991, 1993; Zuckermann, *et al.*, 1992; Sebestyen, *et al.*, 1993).

The identity of library compounds with desired effects on the binding of a Wnt to a Wnt receptor can be determined by conventional means, such as iterative synthesis methods in which sublibraries containing known residues in one subunit position only are identified as containing active compounds.

IX. Pharmaceutical Preparations of Active Compounds

After identifying certain test compounds as potential WntR agonists or antagonists, the practitioner of the screening assay will typically continue to test the efficacy and specificity of the selected compounds both *in vitro* and *in vivo*. Whether for subsequent *in*

vivo testing, or for administration to an animal as an approved drug, agents identified in the screening assay can be formulated in pharmaceutical preparations for *in vivo* administration to an animal, preferably a human.

The compounds selected in the screening assay, or a pharmaceutically acceptable salt thereof, may accordingly be formulated for administration with a biologically acceptable medium, such as water, buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like) or suitable mixtures thereof. The optimum concentration of the active ingredient(s) in the chosen medium can be determined empirically, according to procedures well known to medicinal chemists. As used herein, "biologically acceptable medium" includes any and all solvents, dispersion media, and the like which may be appropriate for the desired route of administration of the pharmaceutical preparation. The use of such media for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the activity of the compound, its use in the pharmaceutical preparation of the invention is contemplated.

Suitable vehicles and their formulation inclusive of other proteins are described, for example, in Gennaro, 1990. These vehicles include injectable "deposit formulations". Based on the above, such pharmaceutical formulations include, although not exclusively, solutions or freeze-dried powders of the compound in association with one or more pharmaceutically acceptable vehicles or diluents, and contained in buffered media at a suitable pH and isosmotic with physiological fluids. In a preferred embodiment, the compound can be disposed in a sterile preparation for topical and/or systemic administration. In the case of freeze-dried preparations, supporting excipients such as, but not exclusively, mannitol or glycine may be used and appropriate buffered solutions of the desired volume will be provided so as to obtain adequate isotonic buffered solutions of the desired pH. Similar solutions may also be used for the pharmaceutical compositions in isotonic solutions of the desired volume and include, but not exclusively, the use of buffered saline solutions with phosphate or citrate at suitable concentrations so as to obtain at all times isotonic pharmaceutical preparations of the desired pH (for example, neutral pH).

The following examples illustrate but in no way are intended to limit the present invention.

MATERIALS AND METHODS

Unless otherwise indicated, restriction enzymes and DNA modifying enzymes were obtained from New England Biolabs (Beverly, MA) or Boehringer Mannheim (Indianapolis, IN). Nitrocellulose paper was obtained from Schleicher and Schuell (Keene, NH). Other chemicals were purchased from Sigma (St. Louis, MO) or United States Biochemical (Cleveland, OH). Unless otherwise specified, the experiments were performed using standard methods (Ausubel, *et al.*, 1988; Sambrook, *et al.*, 1989; Harlow, *et al.*, 1988).

A. Buffers

Phosphate-buffered saline (PBS)
10x stock solution, 1 liter:
80 g NaCl
2 g KCl
11.5 g $Na_2HPO4\text{-}7H_2O$
2 g KH_2PO_4
Working solution, pH 7.3:
137 mM NaCl
2.7 mM KCl
4.3 mM $Na_2HPO_4\text{-}7H_2O$
1.4 mM KH_2PO_4

EXAMPLE 1

Molecular Cloning of DFz2

Polymerase chain reaction (PCR; Mullis, 1987; Mullis, *et al.*, 1987) primer pools YW157 and YW158 were designed based on sequences (SEQ ID NO:16, SEQ ID NO:17, respectively) conserved in Dfz1, Human frizzled 3 (Hfz3), Rat frizzled 1 (Rfz1) and Rat frizzled 2 (Rfz2). The primer pools were completely degenerate, that is, each possible codon of each amino acid in SEQ ID NO:16 and SEQ ID NO:17 was represented in the respective primer pool, with the exception that the wobble base of the 3'-most codon was not included in YW157. The primers were used to amplify *Drosophila* genomic DNA, resulting in an amplification product that, when sequenced, was found to contain a novel *frizzled* family member - Dfz2. The PCR product was used to isolate genomic clones of Dfz2 from an adult *Drosophila* genomic library (Maniatis, *et al.*) and cDNA clones from a 0-24 hr cDNA library.

The amino acid sequence of Dfz2 was compared to that of Dfz1 by aligning the sequences as shown in Fig. 1. Dfz2 and Dfz1 are 32% identical. Identical residues are

indicated in the consensus and the conserved cysteine residues in the cysteine-rich domain are in bold-face. The sequence alignments were done using the "GENEWORKS" program.

Hydropathy values were calculated using the "MACVECTOR" 3.5 software according to the Kyte-Doolittle software and a window size of 15 amino acids.

EXAMPLE 2

In Situ RNA Hybridization

In situ hybridization experiments were performed to determine the pattern of Dfz2 expression. Freshly dissected adult brains, whole embryos or heads were rapidly frozen in plastic molds placed on a dry ice/alcohol slurry and processed for sectioning as described previously (Cole, *et al.*, 1990). ^{35}S-Labeled antisense riboprobes were prepared from linearized p"BLUESCRIPT" plasmid subclones using either T3 or T7 RNA polymerase. In situ hybridization was performed as described by Saffen, *et al.*, and hybridized sections were exposed to X-ray film and digitized.

EXAMPLE 3

Expression of DFz2 During *Drosophila* Development

The expression pattern of DFz2 was assessed using Northern (RNA) blot analysis. Total RNA was isolated using the LiCl-Urea precipitation method (Auffray and Rougeon, 1980). 30 microgram of RNA from each sample was resolved on a formaldehyde 1% agarose gel. The RNA was transferred to a nylon filter, cross-linked by UV irradiation and hybridized to a probe made by random priming Dfz2 or RP49 DNA fragments using standard methods (Sambrook, *et al.*, 1989). In other experiments, Poly (A)$^+$ RNA from various stages of Drosophila development was first selected from total RNA using the Invitrogen "FASTTRACK" 2.0 kit and 5 μg was loaded per lane.

Exemplary results are shown in Figure 3. A 4.0 kb transcript was detected in embryonic stages 0-2; 2-3; 4-5; 9-12, first, second and third instar larvae and pupae. A transcript of similar size was observed in *Drosophila* clone-8 cells (cl-8), a cell line from imaginal discs previously shown to be responsive to Wg activity *in vitro*. *Drosophila* Schneider 2 (S2) cells, which do not respond to Wg, did not contain detectable DFz2 transcripts. The blot was also probed for expression of the ribosomal protein RP49 (O'Connell and Rosbash, 1994, lower panel) as a control for RNA integrity and loading.

EXAMPLE 4

Transfection of DFz2 in S2 Cells Confers a Response to Wg protein

S2 cells were evaluated for Dfz2 expression because the cells are known not to respond to Wg (Yanagawa, *et al.*, 1995). Since, as described above, the native cells did not express Dfz2, they were used in Dfz2 transfection experiments to determine whether expression of Dfz2 would confer sensitivity to Wg.

An expression vector containing DFz2 coding sequences under the control of a metal-inducible metallothionein promoter was used to transfect S2 cells using standard methods. Stable cell lines were derived by selection in hygromycin and tested for Dfz2 expression. In cells grown in the absence of inducers, a baseline level of expression was detected with an antiserum to Dfz2. Induction of the metallothionein promoter resulted in increased levels of expression.

Sensitivity of the Dfz2-transfected S2 cells to Wg protein was assessed by measuring the levels of armadillo (Arm) protein in response to Wg application. In intact Drosophila embryos and in clone-8 cells, Arm protein migrates in two different forms, differing from each other in phosphorylation. When these cells are incubated in the presence of soluble Wg protein, the level of the faster migrating (non-phosphorylated) form increases (Peifer, *et al.*, 1994; Riggleman, *et al.*, 1990; Van Leeuwen, *et al.*, 1994). This increase can be detected using a standard Western blot assay as described below.

Conditioned medium containing Wg protein was produced by subjecting S2*HSwg* cells to heat-shock for 30 minutes at 37°C, allowing the cells to recover for 30 minutes at 25°C, and resuspending them in S2 medium without fetal calf serum (FCS). The cells were incubated for 3 hrs to allow secretion of proteins into the medium, after which they were removed by centrifugation (10 min., 2000 xg and 1hr, 100,000 xg, respectively). The conditioned media were concentrated 12-fold ("CENTRIPREP30", Amicon) and used in the experiments as follows.

Clone 8, untransformed S2, and Dfz-transformed S2 (S2*Dfz2*) cells were incubated for 2 hrs in 6-well dishes in either normal concentrated medium or in concentrated medium from S2 cells producing Wg.

Overexpression of the *Dfz2* gene (under control of the metallothionein promoter) was induced by culturing S2*Dfz2* and S2 control cells in S2 medium containing 0.5 mM $CuSO_4$ for 5 hrs prior to the incubation with the conditioned media.

The target cells were lysed in lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1% Nonidet-P40, 5 mM EDTA) supplemented with 20 μg leupeptin, 100 μg aprotinin and

180 μg PMSF per ml. The extracts were subjected to electrophoresis and Western blotting. Blots were stained in Ponceau Red to evaluate equal loading of total protein and transfer, and then incubated overnight in blocking buffer with monoclonal anti-arm antibody 7A1 at a 1:1000 dilution or rat-polyclonal anti- α-catenin antibody DCAT-1 (Oda, *et al.*, 1993), diluted 1:1000. The blots were washed three times for 15 min each in TBST and incubated for 1 hr with horseradish peroxidase conjugated secondary antibodies (Biorad) diluted 1:20,000 in blocking buffer.

Incubation of DFz2-transfected S2 cells (but not untransfected S2 cells) in the presence of soluble Wg protein resulted in an increase in the level of Arm protein similar to that observed in *Drosophila* embryos and clone-8 cells. Exemplary results are shown in Fig. 4. Addition of Wg (wingless) results in increased signal intensity of the armadillo band. No such effect is observed with untransfected S2 cells. However, all four independent Dfz2-transfected S2 cell lines, derived from two separate transfections, showed increased armadillo signal in response to Wg (two of the four are shown). Further induction of Dfz2 expression by copper sulphate in the transfected cells led to a slight decrease in the response to Wg. As a control for equal loading, the blots were stripped and incubated with an antiserum against α-catenin (lower panel).

EXAMPLE 5

Wg Protein Binds to Dfz2 Transfected Cells

The results described in Example 4 showed that Dfz2 acts as a signal transducing molecule for Wg, suggesting that it is a receptor for Wg. Immunohistochemical analyses were performed to determine whether Wg was capable of binding to the Dfz2-transfected cells.

Nontransfected Sneider 2 (S2) cells and S2 cells expressing Dfz2 were washed twice in PBS and incubated with 1.5 ml of medium alone or 1.5 ml of a 10x concentrated stock of Wg conditioned medium at 4°C for 3 hours. After three 10 minute washes with PBS, the cells were fixed in 2% methanol-free formaldehyde (Polysciences, Inc) for 15 minutes at room temperature. Following three more 10 minute washes with PBS, affinity purified Wg antibody at 1/25 and 5% donkey serum were added to the cells in PBS and incubated overnight at 4°C.

The antiserum was affinity-purified using a bacterial fusion protein containing a domain unique to Wg (the Wg insert -- an 85 amino acid sequence not found in any wg orthologs). Previous experiments have indicated that this domain is dispensable for Wg

activity, that it probably does not participate in the interactions between Wg and its receptor.

Following 3 additional 10 minute washes, fluorescent-labeled cy3 secondary antibody, donkey anti-rabbit (Sigma), at 1/100 and 5% donkey serum were added to the cells for 1 hour at room temperature. The cells were then washed 3 more times in PBS and mounted in Vectashield mounting medium (Vector).

Confocal images were collected with a Bio-Rad MRC 1000 confocal laser attached to a Zeiss Axio scope microscope. Exemplary images are shown in Figs 5A-5F. Normal and transfected cells were incubated with either normal S2 medium (Fig. 5A) or concentrated conditioned medium from S2 cells producing Wg (Figs. 5B, 5C, 5D, 5E, 5F). Dfz2-transfected S2 cells stained brightly in approximately 80% of the cells when incubated with Wg and the antiserum (Figure 5D) whereas Dfz2-transfected cells in the absence of Wg protein (Fig. 5A) as well as non transfected S2 cells (Fig. 5B) showed only some spots of background staining. The positive staining was not uniform over the cell surface but punctate and may reflect clustering of receptor complexes.

The ability of Wg to bind was also tested in heterologous cells (human 293 cells) transiently-transfected with Dfz2. In view of high background binding observed in initial experiments, the transiently-transfected 293 cells were preincubated with chlorate, which inhibits sulfation of proteins and glucosaminoglycans, and with heparatinase, to remove heparin-like molecules. This pre-treatment significantly lowered the background binding (presumably due to Wg binding to extracellular matrix; Fig. 5E). As shown in Fig. 5F, about 10-20% of the transfected cells remained positive, similar to the transfection efficiency of 293 cells. Since 293 cells are of human origin, these results strongly suggest that Wg binds to Dfz2 itself, rather than to a molecule whose expression is induced by Dfz2.

In contrast to the positive staining patterns observed with Dfz2-transfected cells, no staining was detected in S2 cells expressing Notch (Fig. 5C). Notch is a protein that has been previously proposed to act as a receptor for Wg (Couso and Arias, 1994).

The above results taken together indicate that Wg protein can specifically bind to cells expressing Dfz2, and that this binding is not likely due to clonal variation.

EXAMPLE 6

Binding of Metabolically-Labeled Wg Protein to a Dfz-2/IgG Fusion Protein

The binding of Wg protein to Dfz2 itself was also assayed using a fusion protein containing the cysteine-rich amino-terminal domain of Dfz2, linked to the constant domain of human IgG. The fusion protein or IgG control was added to conditioned medium from normal S2 cells, or S2 cells producing Wg (HS-wg/S2), which had been metabolically-labeled with [^{35}S] cysteine and methionine.

The fusion proteins and possible complexes were then retrieved by adding sepharose-ProteinA beads and analyzed by gel electrophoresis and fluorography. Figure 6 shows that the Dfz2 fusion protein, but not the control IgG, selectively binds to labeled proteins of 52 kD, the size of the mature Wg protein. Normal S2 cells did not produce Dfz-2 binding proteins.

While the invention has been described with reference to specific methods and embodiments, it is appreciated that various modifications and changes may be made without departing from the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: The Board of Trustees of the Leland Stanford Junior University, *et al.*

(ii) TITLE OF INVENTION: Wnt Receptor Compositions and Methods

(iii) NUMBER OF SEQUENCES: 18

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 11-APR-1997
(C) CLASSIFICATION:

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2344 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Dfz2 Polynucleotide, coding region begins at nucleotide #225

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCGCTGTGTC TGAAGGAAAC ACTACCCGCT TTTCCGGCTC TCGAGGCGCC TCCACGAAGG 60

AGTGAGGTGC AACCGCAGAG AAGGTCAGCA AAGAAAGAGC AAGGGGTTCC AAGTCACACA 120

ACCGAACTAA GCTAAGACGC ACAAAATGAG ACACAATCGA CTGAAGGTCC TGATCCTGGG 180

ACTCGTCCTC CTGCTGACAT CTTGTCGAGC GGATGGACCG CTGCACAGTG CGGATCACGG 240

CATGGGCGGA ATGGGCATGG GTGGTCACGG CCTGGACGCG AGTCCCGCAC CCGGTTACGG 300

AGTGCCAGCC ATACCCAAGG ATCCCAATCT GCGATGCGAG GAGATCACCA TACCAATGTG 360

TCGGGGCATT GGCTACAACA TGACATCCTT CCCCAACGAA ATGAACCATG AGACCCAGGA 420

CGAAGCGGGC CTGGAGGTGC ACCAGTTCTG GCCCCTGGTG GAGATCAAAT GCTCGCCGGA 480

CCTCAAGTTC TTCCTGTGCA GCATGTACAC GCCCATCTGC CTGGAGGATT ACCACAAGCC 540

GCTGCCCGTT TGCCGGAGTG TCTGCGAGAG AGCCCGCTCG GGATGCGCAC CCATCATGCA 600

GCAGTACAGC TTCGAATGGC CGGAGAGAAT GGCGTGCGAG CACTTGCCCC TTCATGGTGA 660

CCCCGACAAT CTGTGCATGG AACAGCCCTC GTACACGGAG GCTGGCAGCG GTGGCAGCTC 720

GGGCGGATCG GGTGGCTCTG GCAGCGGTTC CGGCTCCGGC GGCAAACGGA AGCAAGGAGG 780

CAGTGGCTCG GGCGGCAGTG GGGCCGGCGG CAGCAGCGGT TCCACCTCAA CGAAGCCGTG 840

CCGCGGACGC AATTCAAAAA ACTGCCAAAA TCCCCAAGGA GAAAAGGCAA GCGGAAAAGA 900

GTGCAGCTGC TCGTGCCGCT CCCCACTCAT CTTCCTGGGG AAGGAGCACT GGCTGCAGCA 960

GCAGTCGCAG ATGCCCATGA TGCACCATCC ACACCACTGG TACATGAACC TCACTGTCCA 1020

AAGGATCGCC GGCGTTCCAA ACTGCGGCAT ACCGTGCAAG GGGCCCTTCT TCAGCAACGA 1080

CGAAAAGGAT TTCGCCGGCC TCTGGATCGC CCTGTGGTCG GGACTGTGCT TCTGCAGCAC 1140

GCTCATGACC CTAACCACAT TCATCATCGA CACCGAAAGG TTTAAGTACC CGGAGCGGCC 1200

ATTGTCTTCC TCTCCGCCTG CTACTTCATG GTGGCAGTGG GCTACCTGTC GCGCAACTTC 1260

CTGCAGAACG AGGAGATCGC CTGCGACGGC CTGCTGCTCC GGGAAAGCTC CACGGGTCCG 1320

CACTCTTGCA CCCTGGTCTT CCTGCTCACC TACTTCTTTG GCATGGCCTC GTCCATCTGG 1380

TGGGTGATCC TCACTTTCAC CTGGTTCCTG GCCGCTGGTC TGAAGTGGGG CAATGAGGCC 1440

ATCACCAAGC ACTCGCAGTA CTTCCATCTG GCCGCCTGGT TGATTCCCAC TGTCCAGTCC 1500

GTGGCCGTAC TCCTGCTCTC GGCGGTGGAT GGCGATCCCA TTCTGGGCAT CTGCTATGTG 1560

GGCAACCTCA ATCCGGATCA CCTAAAGACC TTTGTGCTGG CCCCGCTCTT AGTTTACCTC 1620

GTAATCGGCA CCACCTTCCT GATGGCCGGC TTTGTGTCCC TCTTCCGCAT CCGCTCGGTT 1680

ATCAAGCAAC AGGGCGGTGT AGGAGCTGGT GTCAAGGCGG ACAAGCTGGA GAAACTGATG 1740

ATCAGGATTG GCATCTTCTC GGTGCTCTAC ACGGTGCCGG CCACCATAGT TATCGGATGT 1800

TACCTGTACG AAGCAGCCTA CTTTGAGGAC TGGATCAAGG CCCTGGCCTG TCCATGCGCC 1860

CAGGTGAAGG GTCCCGGCAA GAAGCCTCTC TACTCGGTCC TGATGCTCAA GTACTTCATG 1920

GCCCTGGCCG TGGGCATCAC CTCGGGCGTG TGGATCTGGT CTGGCAAGAC GCTGGAGAGC 1980

TGGCGACGCT TCTGGCGGAG ACTCCTAGGA GCGCCGGACC GCACGGGCGC CAACCAGCTG 2040

GCGATCAAGC AGCGGCCTCC GATCCCGCAT CCCTATGCCG GATCTGGAAT GGGCATGCCC 2100

GTGGGCTCGG CGGCGGGCTC CCTGCTGGCC ACGCCCTACA CCCAGGCGGG CGGACGTCGG 2160

TGGCCTCCAC CAGCCACCAC CACCTGCACC ACCACGTTCT CAAGCAGCCG GCGGCCAGCC 2220

ACGTATGACA TGGAGAGTCG GGGGGAGCAT CGACCATGGG CGGCGGTGGG GGCGGCGGTA 2280

CAGCCCTTGG CGGCGGCACC CTGGGCCACG GCACCGCGAT GAGCAGCAGC ACGGTCGGCA 2340

TGGG 2344

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 694 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Dfz2 Polypeptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Arg His Asn Arg Leu Lys Val Leu Ile Leu Gly Leu Val Leu Leu
1 5 10 15

Leu Thr Ser Cys Arg Ala Asp Gly Pro Leu His Ser Ala Asp His Gly
20 25 30

Met Gly Gly Met Gly Met Gly Gly His Gly Leu Asp Ala Ser Pro Ala
35 40 45

Pro Gly Tyr Gly Val Pro Ala Ile Pro Lys Asp Pro Asn Leu Arg Cys
50 55 60

Glu Glu Ile Thr Ile Pro Met Cys Arg Gly Ile Gly Tyr Asn Met Thr
65 70 75 80

Ser Phe Pro Asn Glu Met Asn His Glu Thr Gln Asp Glu Ala Gly Leu
85 90 95

Glu Val His Gln Phe Trp Pro Leu Val Glu Ile Lys Cys Ser Pro Asp
100 105 110

Leu Lys Phe Phe Leu Cys Ser Met Tyr Thr Pro Ile Cys Leu Glu Asp
115 120 125

Tyr His Lys Pro Leu Pro Val Cys Arg Ser Val Cys Glu Arg Ala Arg
130 135 140

Ser Gly Cys Ala Pro Ile Met Gln Gln Tyr Ser Phe Glu Trp Pro Glu
145 150 155 160

Arg Met Ala Cys Glu His Leu Pro Leu His Gly Asp Pro Asp Asn Leu
165 170 175

Cys Met Glu Gln Pro Ser Tyr Thr Glu Ala Gly Ser Gly Gly Ser Ser
180 185 190

Gly Gly Ser Gly Gly Ser Gly Ser Gly Ser Gly Ser Gly Gly Lys Arg

195 200 205

Lys Gln Gly Gly Ser Gly Ser Gly Gly Ser Gly Ala Gly Gly Ser Ser
210 215 220

Gly Ser Thr Ser Thr Lys Pro Cys Arg Gly Arg Asn Ser Lys Asn Cys
225 230 235 240

Gln Asn Pro Gln Gly Glu Lys Ala Ser Gly Lys Glu Cys Ser Cys Ser
245 250 255

Cys Arg Ser Pro Leu Ile Phe Leu Gly Lys Glu Gln Leu Leu Gln Gln
260 265 270

Gln Ser Gln Met Pro Met Met His His Pro His His Trp Tyr Met Asn
275 280 285

Leu Thr Val Gln Arg Ile Ala Gly Val Pro Asn Cys Gly Ile Pro Cys
290 295 300

Lys Gly Pro Phe Phe Ser Asn Asp Glu Lys Asp Phe Ala Gly Leu Trp
305 310 315 320

Ile Ala Leu Trp Ser Gly Leu Cys Phe Cys Ser Thr Leu Met Thr Leu
325 330 335

Thr Thr Phe Ile Ile Asp Thr Glu Arg Phe Lys Xaa Pro Gly Ala Ala
340 345 350

Ile Val Phe Leu Ser Ala Cys Tyr Phe Met Val Ala Val Gly Tyr Leu
355 360 365

Ser Arg Asn Phe Leu Gln Asn Glu Glu Ile Ala Cys Asp Gly Leu Leu
370 375 380

Leu Arg Glu Ser Ser Thr Gly Pro His Ser Cys Thr Leu Val Phe Leu
385 390 395 400

Leu Thr Tyr Phe Phe Gly Met Ala Ser Ser Ile Trp Trp Val Ile Leu
405 410 415

Thr Phe Thr Trp Phe Leu Ala Ala Gly Leu Lys Trp Gly Asn Glu Ala
420 425 430

Ile Thr Lys His Ser Gln Tyr Phe His Leu Ala Ala Trp Leu Ile Pro
435 440 445

Thr Val Gln Ser Val Ala Val Leu Leu Leu Ser Ala Val Asp Gly Asp
450 455 460

Pro Ile Leu Gly Ile Cys Tyr Val Gly Asn Leu Asn Pro Asp His Leu
465 470 475 480

Lys Thr Phe Val Leu Ala Pro Leu Phe Val Tyr Leu Val Ile Gly Thr
485 490 495

Thr Phe Leu Met Ala Gly Phe Val Ser Leu Phe Arg Ile Arg Ser Val
500 505 510

Ile Lys Gln Gln Gly Gly Val Gly Ala Gly Val Lys Ala Asp Lys Leu
515 520 525

Glu Lys Leu Met Ile Arg Ile Gly Ile Phe Ser Val Leu Tyr Thr Val
530 535 540

Pro Ala Thr Ile Val Ile Gly Cys Tyr Leu Tyr Glu Ala Ala Tyr Phe
545 550 555 560

Glu Asp Trp Ile Lys Ala Leu Ala Cys Pro Cys Ala Gln Val Lys Gly
565 570 575

Pro Gly Lys Lys Pro Leu Tyr Ser Val Leu Met Leu Lys Tyr Phe Met
580 585 590

Ala Leu Ala Val Gly Ile Thr Ser Gly Val Trp Ile Trp Ser Gly Lys
595 600 605

Thr Leu Glu Ser Trp Arg Arg Phe Trp Arg Arg Leu Leu Gly Ala Pro
610 615 620

Asp Arg Thr Gly Ala Asn Gln Ala Leu Ile Lys Gln Arg Pro Pro Ile
625 630 635 640

Pro His Pro Tyr Ala Gly Ser Gly Met Gly Met Pro Val Gly Ser Ala
645 650 655

Ala Gly Ser Leu Leu Ala Thr Pro Tyr Thr Gln Ala Gly Gly Ala Ser
660 665 670

Val Ala Ser Thr Ser His His His Leu His His His Val Leu Lys Gln
675 680 685

Pro Ala Ala Ser His Val
690

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2624 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Mus musculus frizzled-3 protein,
Coding Region: 313..2313

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAATTCGGCA CGAGAAGATG GAATCTGTGA TTTGGGAATG CGGTTGATGG AGTTGCTATG 60

CTGGCCAGAT GTGCCCAATG TAATAAAATG AAAAGAAGAT ACAAGATGAT GTCATCTTCC 120

CATATTGTGA AACCAAAAAC AAATGCCCTT TGTGAGACCA GGTTACCAGT TCTTTGACAG 180

TACAGGGAGT TTTTAAACTG AGGAGCCTAA CAGATAAGGG GTACTTTCAA GCTGAGACCT 240

GCAGGCATAT ACTGATCTAA AACGCATCTT GTGTAGATCT GATCATCCGA GCCTCATTCT 300

GATCCAGGAA GAATGGCTGT GAGCTGGATT GTCTTTGATC TTTGGCTCTT GACTGTGTTT 360

CTGGGGCAGA TAGGTGGGCA CAGTTTGTTT TCTTGTGAAC CTATAACCTT GAGGATGTGC 420

CAAGATTTGC CTTACAATAC TACCTTCATG CCTAATCTTC TGAACCATTA TGACCAACAG 480

ACTGCAGCTT TAGCAATGGA GCCCTTCCAC CCTATGGTGA ACCTGGATTG TTCTCGGGAT 540

TTTCGGCCAT TTCTTTGTGC ACTCTATGCC CCTATTTGTA TGGAATATGG ACGTGTCACA 600

CTTCCCTGCC GTAGGCTGTG TCAGCGTGCC TATAGCGAGT GTTCAAAACT CATGGAGATG 660
TTTGGTGTCC CGTGGCCTGA AGATATGGAG TGCAGTAGGT TTCCAGATTG TGATGAGCCA 720
TATCCCCGAC TTGTGGATTT GAATTTAGTT GGAGATCCAA CTGAAGGAGC CCCAGTTGCA 780
GTGCAGAGGG ACTATGGTTT TTGGTGTCCC AGAGAGTTAA AAATTGATCC TGATCTTGGC 840
TATTCCTTTC TGCACGTGCG AGATTGTTCG CCACCATGTC CCAATATGTA CTTCAGGAGA 900
GAAGAACTGT CATTTGCTCG CTATTTCATA GGCCTGATTT CAATCATTTG CCTCTCTGCC 960
ACATTGTTTA CTTTTTTAAC CTTTCTAATT GACGTCACAA GATTCCGTTA CCCTGAAAGA 1020
CCTATCATAT TTTATGCAGT CTGCTACATG ATGGTGTCAT TAATTTTCTT CATTGGGTTT 1080
TTGCTGGAGG ACCGAGTAGC CTGCAATGCA TCTAGCCCTG CACAGTATAA GGCTTCTACA 1140
GTGACACAAG GATCTCACAA TAAGGCCTGT ACCATGCTCT TTATGGTACT ATATTTTTTC 1200
ACTATGGCTG GCAGTGTATG GTGGGTAATT CTTACCATCA CATGGTTTTT AGCAGCTGTG 1260
CCAAAGTGGG GCAGTGAAGC TATTGAGAAG AAAGCATTGC TGTTTCATGC CAGTGCCTGG 1320
GGCATCCCCG GAACTCTAAC TATCATCCTT TTAGCGATGA ATAAAATTGA AGGTGACAAT 1380
ATTAGTGGCG TGTGTTTTGT CGGCCTCTAC GACGTTGATG CATTAAGATA TTTCGTTCTC 1440
GCTCCCCTCT GCCTGTATGT GGTAGTTGGG GTTTCTCTCC TTTTAGCCGG CATTATATCC 1500
CTAAACAGAG TTCGGATTGA GATCCCATTA GAAAAGGAAA ACCAAGATAA GTTAGTGAAG 1560
TTCATGATCC GGATTGGTGT TTTCAGCATT CTCTACCTTG TGCCACTCTT GGTTGTAATT 1620
GGATGTTACT TTTATGAGCA AGCTTACCGC GGCATCTGGG AGACAACATG GATCCAGGAA 1680
CGCTGCAGAG AGTATCACAT TCCATGTCCG TACCAGGTTA CTCAGATGAG TCGTCCAGAC 1740
CTGATTCTCT TTCTGATGAA GTATCTCATG GCTCTCATAG TTGGGATTCC CTCTATATTT 1800
TGGGTTGGAA GCAAAAAGAC ATGCTTTGAA TGGGCCAGTT TTTTCCATGG GCGTAGGAAA 1860
AAAGAGATAG TGAATGAGAG CCGGCAGGTG CTCCAGGAAC CTGACTTTGC TCAGTCACTC 1920
CTGAGGGACC CAAATACTCC AATTATAAGA AAATCAAGAG GAACTTCCAC TCAAGGGACA 1980
TCCACACATG CTTCTTCAAC TCAGCTGGCC ATGGTGGATG ACCAAAGAAG CAAAGCAGGG 2040
AGTGTCCACA GCAAAGTGAG CAGCTACCAT GGCAGCCTCC ACAGGTCACG GGATGGCAGG 2100
TACACTCCCT GCAGTTACCG AGGAATGGAG GAGAGACTAC CTCACGGCAG CATGTCACGG 2160
CTGACGGATC ATTCCAGGCA CAGTAGTTCT CATCGGCTCA ACGAGCAGTC CCGACACAGC 2220
AGCATCCGAG ACCTCAGTAA CAACCCCATG ACTCACATTA CACATGGCAC CAGCATGAAC 2280
CGTGTTATTG AGGAGGATGG AACCAGTGCT TAGTCTTGTC TAAGGTGAAA TGTGTGCTGT 2340
TGAAAAGCAG GTTTTGCCTT CGCATGGCTG GCTGCTGTAA CTCACTGTCG CTCTGCTTTC 2400
TTGGGCAGAG TGTCAGCCTG GGAAAGTAGA TCTTTGCTCT TTGTATCACA TCAACCCTGG 2460
GGTGTGAACA CATCCAAACC CTAAGGATCA TGTCATCACA AAAGTAATTC TTTCTAGGCT 2520
GTGAAGAGAT GATTGTCTGG TGAGCATTTT TTATAAACAT GCTTATTTTA TATCTAGAAA 2580
AATCCTCTAT GTGTGGTGAC TGCTTTGTAG TGAATTTCAT ATAA 2624

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 667 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Mfz3 protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Val Ser Trp Ile Val Phe Asp Leu Trp Leu Leu Thr Val Phe
1 5 10 15
Leu Gly Gln Ile Gly Gly His Ser Leu Phe Ser Cys Glu Pro Ile Thr
20 25 30
Leu Arg Met Cys Gln Asp Leu Pro Tyr Asn Thr Thr Phe Met Pro Asn
35 40 45
Leu Leu Asn His Tyr Asp Gln Gln Thr Ala Ala Leu Ala Met Glu Pro
50 55 60
Phe His Pro Met Val Asn Leu Asp Cys Ser Arg Asp Phe Arg Pro Phe
65 70 75 80
Leu Cys Ala Leu Tyr Ala Pro Ile Cys Met Glu Tyr Gly Arg Val Thr
85 90 95
Leu Pro Cys Arg Arg Leu Cys Gln Arg Ala Tyr Ser Glu Cys Ser Lys
100 105 110
Leu Met Glu Met Phe Gly Val Pro Trp Pro Glu Asp Met Glu Cys Ser
115 120 125
Arg Phe Pro Asp Cys Asp Glu Pro Tyr Pro Arg Leu Val Asp Leu Asn
130 135 140
Leu Val Gly Asp Pro Thr Glu Gly Ala Pro Val Ala Val Gln Arg Asp
145 150 155 160
Tyr Gly Phe Trp Cys Pro Arg Glu Leu Lys Ile Asp Pro Asp Leu Gly
165 170 175
Tyr Ser Phe Leu His Val Arg Asp Cys Ser Pro Pro Cys Pro Asn Met
180 185 190
Tyr Phe Arg Arg Glu Glu Leu Ser Phe Ala Arg Tyr Phe Ile Gly Leu
195 200 205
Ile Ser Ile Ile Cys Leu Ser Ala Thr Leu Phe Thr Phe Leu Thr Phe
210 215 220
Leu Ile Asp Val Thr Arg Phe Arg Tyr Pro Glu Arg Pro Ile Ile Phe
225 230 235 240
Tyr Ala Val Cys Tyr Met Met Val Ser Leu Ile Phe Phe Ile Gly Phe
245 250 255

Leu Leu Glu Asp Arg Val Ala Cys Asn Ala Ser Ser Pro Ala Gln Tyr
260 265 270

Lys Ala Ser Thr Val Thr Gln Gly Ser His Asn Lys Ala Cys Thr Met
275 280 285

Leu Phe Met Val Leu Tyr Phe Phe Thr Met Ala Gly Ser Val Trp Trp
290 295 300

Val Ile Leu Thr Ile Thr Trp Phe Leu Ala Ala Val Pro Lys Trp Gly
305 310 315 320

Ser Glu Ala Ile Glu Lys Lys Ala Leu Leu Phe His Ala Ser Ala Trp
325 330 335

Gly Ile Pro Gly Thr Leu Thr Ile Ile Leu Leu Ala Met Asn Lys Ile
340 345 350

Glu Gly Asp Asn Ile Ser Gly Val Cys Phe Val Gly Leu Tyr Asp Val
355 360 365

Asp Ala Leu Arg Tyr Phe Val Leu Ala Pro Leu Cys Leu Tyr Val Val
370 375 380

Val Gly Val Ser Leu Leu Leu Ala Gly Ile Ile Ser Leu Asn Arg Val
385 390 395 400

Arg Ile Glu Ile Pro Leu Glu Lys Glu Asn Gln Asp Lys Leu Val Lys
405 410 415

Phe Met Ile Arg Ile Gly Val Phe Ser Ile Leu Tyr Leu Val Pro Leu
420 425 430

Leu Val Val Ile Gly Cys Tyr Phe Tyr Glu Gln Ala Tyr Arg Gly Ile
435 440 445

Trp Glu Thr Thr Trp Ile Gln Glu Arg Cys Arg Glu Tyr His Ile Pro
450 455 460

Cys Pro Tyr Gln Val Thr Gln Met Ser Arg Pro Asp Leu Ile Leu Phe
465 470 475 480

Leu Met Lys Tyr Leu Met Ala Leu Ile Val Gly Ile Pro Ser Ile Phe
485 490 495

Trp Val Gly Ser Lys Lys Thr Cys Phe Glu Trp Ala Ser Phe Phe His
500 505 510

Gly Arg Arg Lys Lys Glu Ile Val Asn Glu Ser Arg Gln Val Leu Gln
515 520 525

Glu Pro Asp Phe Ala Gln Ser Leu Leu Arg Asp Pro Asn Thr Pro Ile
530 535 540

Ile Arg Lys Ser Arg Gly Thr Ser Thr Gln Gly Thr Ser Thr His Ala
545 550 555 560

Ser Ser Thr Gln Leu Ala Met Val Asp Asp Gln Arg Ser Lys Ala Gly
565 570 575

Ser Val His Ser Lys Val Ser Ser Tyr His Gly Ser Leu His Arg Ser
580 585 590

Arg Asp Gly Arg Tyr Thr Pro Cys Ser Tyr Arg Gly Met Glu Glu Arg
595 600 605

Leu Pro His Gly Ser Met Ser Arg Leu Thr Asp His Ser Arg His Ser

610 615 620

Ser Ser His Arg Leu Asn Glu Gln Ser Arg His Ser Ser Ile Arg Asp
625 630 635 640

Leu Ser Asn Asn Pro Met Thr His Ile Thr His Gly Thr Ser Met Asn
645 650 655

Arg Val Ile Glu Glu Asp Gly Thr Ser Ala Glx
660 665

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1770 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Caenorhabditis elegans putative transmembrane receptor (frizzled 1) gene,
Coding region: 57..1634

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAATTCGGTT TAATTACCCA AGTTTGAGCT GTGAGCCCCC AATTCCATTA TCATTAATGG 60

GACCATTTCG TGGTTACCTC GGAGTAACCT GGCTCCTGTT GCTCTTTGTG ATTGGTGTGG 120

ACGGGCAGAG GTGTCAAAAG GTGGATCATG AGATGTGCAA CGATTTGCCG TATAACTTAA 180

CGAGCTTCCC AAATCTCGTC GACGAGGAAT CATGGAAAGA CGCCTCCGAA TCCATCCTCA 240

CCTACAAGCC CCTGCTCTCC GTTGTCTGCT CCGAGCAGCT CAAATTCTTC CTGTGCTCCG 300

TCTACTTCCC GATGTGCAAC GAGAAACTAG CCAACCCAAT TGGTCCATGC CGTCCATTGT 360

GTCTTTCCGT CCAGGAAAAG TGTCTTCCAG TGCTGGAAAG TTTCGGTTTC AAGTGGCCCG 420

ATGTGATTCG TTGTGATAAG TTCCCGTTGG AGAACAATCG AGAGAAAATG TGCATGAAAG 480

GGCCAAATGA GCAAGGAGCA ATTCAAGATG AGAGGGCAAA GTTTGCAGCG AAAGAAAGTG 540

AGGACGACGG TAATGATCGA GTAGAAGATA TTCAACGGGA GGTCGACCGC CTCAACGGAA 600

AATGCCCACA GGATGAGGTG TTCCTGAATC GATCCTCAAA GTGTGTGCCT TTGTGCTCGA 660

ACCCACAGAA GGTTGGGCAG ACTGACCGTG AATCCGCCAC CCGACTCTTG TTGTTTCTCT 720

CGCTGAGCTC TGTAATACTA ACAATTCTAT CAGTCTTCAT AGTCGGCTTA TCACGTCTCG 780

AGATGCTCCA CTCACTTACG GAAACTGCCA TGTTCTTCTC GTGCATCTCG TTTTGTGCGA 840

CATCGGTTAT TTATATTGTG AGCATTTCGT TTAAAGATCA GTTCCAAATC TCGTGCACCG 900

ACTACACCCA TCACCTGCTC TTCGTCGTCG GAGGGCTTTC CCATGTTCCA TGTTCTTCAG 960

TGGCCTCACT GATTTACTAC ACGGCAACTT GCTCACGTCT CTGGTGGCTC TTGATCTGTG 1020

TGTCGTGGAA TAAGGCGACA AGGACATCGC ATATATTGGA CGACTCCAGA ACCCGCGTGA 1080

TCATGCTCAT CCTGGGAATC CCGCTGGCTC CACTAATGCT CGCGCTACTC GCAAAAGCCG 1140

TCGCCGCCAA TCCCCTCACC GGACTCTGCT TCATCGGAGC AGCAAGCCCG GGCACCGACT 1200

GGATCTTCAA CTTCTGCCGG GAGCTCATTC TATTCCTCAT CAGCTCCATT GCTCTTTCGT 1260

CTGCTTGCTG CCGGCTTCTG GGCTCTGATG AGCAGGATGT CAATGGGTTT GCCGGAGTCA 1320

TTGCGGCAGT CTATCCGATT GCTGGACTAT TCTACATGCT TTCATTTGTG AACGATGCCA 1380

CCCAACCGTT TCTCTCACTT GACAGAAGTT TCAATGCGGT CTCGGCGACC AAGTTCTCGT 1440

TTGATCTACT TTTGAGCTTC ATCATGTGCG CGTTTTGTCT TATTTACTTG CTGTTCAAGC 1500

TGACTAGATC CTCATCAAAA GTTAGCAAAG AAGGATATCA ACCGGCGGTG CCGAAACTCC 1560

CGCAACCGGC AATTCCCGGC AGTGTACGTT CGAACACCTA CGCGTCGACG TTTCGAACTA 1620

ATAATATGAT TTGAAGGATT TTCAATAATT TTTTGTGAAA AACAACGGGT TTATATAGAT 1680

AGAAAACAAA AAGGTGGTCT CAATTTTTTT TCCGTGAAAA TAAATTTTTA TTGATTTTTA 1740

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 1770

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 526 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Cfz1 protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Gly Pro Phe Arg Gly Tyr Leu Gly Val Thr Trp Leu Leu Leu Leu
1 5 10 15

Phe Val Ile Gly Val Asp Gly Gln Arg Cys Gln Lys Val Asp His Glu
20 25 30

Met Cys Asn Asp Leu Pro Tyr Asn Leu Thr Ser Phe Pro Asn Leu Val
35 40 45

Asp Glu Glu Ser Trp Lys Asp Ala Ser Glu Ser Ile Leu Thr Tyr Lys
50 55 60

Pro Leu Leu Ser Val Val Cys Ser Glu Gln Leu Lys Phe Phe Leu Cys
65 70 75 80

Ser Val Tyr Phe Pro Met Cys Asn Glu Lys Leu Ala Asn Pro Ile Gly
85 90 95

Pro Cys Arg Pro Leu Cys Leu Ser Val Gln Glu Lys Cys Leu Pro Val
100 105 110

Leu Glu Ser Phe Gly Phe Lys Trp Pro Asp Val Ile Arg Cys Asp Lys
115 120 125

Phe Pro Leu Glu Asn Asn Arg Glu Lys Met Cys Met Lys Gly Pro Asn
130 135 140

Glu Gln Gly Ala Ile Gln Asp Glu Arg Ala Lys Phe Ala Ala Lys Glu
145 150 155 160

Ser Glu Asp Asp Gly Asn Asp Arg Val Glu Asp Ile Gln Arg Glu Val
165 170 175

Asp Arg Leu Asn Gly Lys Cys Pro Gln Asp Glu Val Phe Leu Asn Arg
180 185 190

Ser Ser Lys Cys Val Pro Leu Cys Ser Asn Pro Gln Lys Val Gly Gln
195 200 205

Thr Asp Arg Glu Ser Ala Thr Arg Leu Leu Leu Phe Leu Ser Leu Ser
210 215 220

Ser Val Ile Leu Thr Ile Leu Ser Val Phe Ile Val Gly Leu Ser Arg
225 230 235 240

Leu Glu Met Leu His Ser Leu Thr Glu Thr Ala Met Phe Phe Ser Cys
245 250 255

Ile Ser Phe Cys Ala Thr Ser Val Ile Tyr Ile Val Ser Ile Ser Phe
260 265 270

Lys Asp Gln Phe Gln Ile Ser Cys Thr Asp Tyr Thr His His Leu Leu
275 280 285

Phe Val Val Gly Gly Leu Ser His Val Pro Cys Ser Ser Val Ala Ser
290 295 300

Leu Ile Tyr Tyr Thr Ala Thr Cys Ser Arg Leu Trp Trp Leu Leu Ile
305 310 315 320

Cys Val Ser Trp Asn Lys Ala Thr Arg Thr Ser His Ile Leu Asp Asp
325 330 335

Ser Arg Thr Arg Val Ile Met Leu Ile Leu Gly Ile Pro Leu Ala Pro
340 345 350

Leu Met Leu Ala Leu Leu Ala Lys Ala Val Ala Ala Asn Pro Leu Thr
355 360 365

Gly Leu Cys Phe Ile Gly Ala Ala Ser Pro Gly Thr Asp Trp Ile Phe
370 375 380

Asn Phe Cys Arg Glu Leu Ile Leu Phe Leu Ile Ser Ser Ile Ala Leu
385 390 395 400

Ser Ser Ala Cys Cys Arg Leu Leu Gly Ser Asp Glu Gln Asp Val Asn
405 410 415

Gly Phe Ala Gly Val Ile Ala Ala Val Tyr Pro Ile Ala Gly Leu Phe
420 425 430

Tyr Met Leu Ser Phe Val Asn Asp Ala Thr Gln Pro Phe Leu Ser Leu
435 440 445

Asp Arg Ser Phe Asn Ala Val Ser Ala Thr Lys Phe Ser Phe Asp Leu
450 455 460

Leu Leu Ser Phe Ile Met Cys Ala Phe Cys Leu Ile Tyr Leu Leu Phe

Lys Leu Thr Arg Ser Ser Ser Lys Val Ser Lys Glu Gly Tyr Gln Pro Ala Val Pro Lys Leu Pro Gln Pro Ala Ile Pro Gly Ser Val Arg Ser Asn Thr Tyr Ala Ser Thr Phe Arg Thr Asn Asn Met Ile Glx

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2828 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Mus musculus putative transmembrane receptor (frizzled 4) mRNA,
Coding region: 238..1941

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TCGACCTCAA CACAAAGACC TGGGTCGTGA GACACACGCG TAGAGTCAGG CGGCTTCCCC 60
GAAAACCGGA CTCGGCCGGC GCCGAGTCTG GGTCCCCGCC TTCAACCATG ACCCTAGCAA 120
TCCATCCCTC GGCCCGGGCT CCGGACGTCT GATATTCCGC ACATTCTCGT ACAACTGCTG 180
GAGAGGCGAC TGCTGCCCCC TTGTCGCCCT TGGCGCCTTA CCGCATTCCC TATCCGGAGT 240
TGGGAGCAGC GCGGCCACCG GCGCCCCTGT GCAAACTGGG GGTGTCTGCT AGATCAGCCT 300
CTGCCGCTGC TGCCCGCAGC TCTGGCCATG GCCTGGCCGG GCACAGGGCC GAGCAGCCGG 360
GGGGCGCCTG GAGGCGTCGG GCTCAGGCTG GGGCTGCTGC TGCAGTTCCT CCTGCTCCTG 420
CGGCCGACAC TGGGGTTCGG GGACGAGGAG GAGCGGCGCT GCGACCCCAT CCGCATCGCC 480
ATGTGCCAGA ACCTCGGCTA CAACGTGACC AAGATGCCCA ACTTAGTGGG ACACGAGCTG 540
CAGACAGACG CCGAGCTGCA GCTGACAACT TTCACGCCGC TCATCCAGTA CGGCTGCTCC 600
AGCCAGCTGC AGTTCTTCCT TTGTTCGGTT TATGTGCCAA TGTGCACAGA GAAGATCAAC 660
ATCCCCATCG GCCCGTGCGG TGGCATGTGC CTTTCAGTCA AGAGACGCTG TGAACCAGTC 720
CTGAGAGAAT TTGGGTTTGC CTGGCCCGAC ACCCTGAACT GCAGCAAGTT CCCGCCCCAG 780
AACGACCACA ACCACATGTG CATGGAAGGA CCAGGTGATG AAGAGGTTCC CTTGCCCCAC 840
AAGACTCCCA TCCAGCCCGG GGAAGAGTGC CACTCCGTGG GAAGCAATTC TGATCAGTAC 900
ATCTGGGTGA AGAGGAGCCT GAACTGTGTT CTCAAGTGTG GCTACGATGC TGGCTTGTAC 960
AGCCGCTCAG CTAAGGAGTT CACGGATATT TGGATGGCTG TGTGGGCCAG CCTCTGCTTC 1020

ATCTCCACCA CCTTCACCGT GCTGACCTTC CTGATTGATT CATCCAGGTT TTCTTACCCT 1080

GAGCGCCCCA TCATATTTCT CAGTATGTGC TATAATATTT ATAGCATTGC TTATATTGTT 1140

CGGCTGACTG TAGGCCGGGA AAGGATATCC TGTGATTTTG AAGAGGCGGC AGAGCCCGTT 1200

CTCATCCAAG AAGGACTTAA GAACACAGGA TGTGCAATAA TTTTCTTGCT GATGTACTTT 1260

TTTGGAATGG CCAGCTCCAT TTGGTGGGTT ATTCTGACAC TCACTTGGTT TTTGGCAGCC 1320

GGACTCAAGT GGGGTCATGA AGCCATTGAA ATGCACAGTT CTTATTTCCA CATCGCAGCC 1380

TGGGCTATTC CCGCAGTGAA AACCATTGTC ATCTTGATTA TGAGACTAGT GGATGCCGAT 1440

GAACTGACTG GCTTGTGCTA TGTTGGGAAC CAAAACCTAG ATGCCCTCAC TGGCTTTGTG 1500

GTGGCTCCTC TCTTTACGTA TTTGGTGATT GGAACGCTGT TCATTGCGGC GGGTTTGGTG 1560

GCCTTATTCA AAATTCGGTC CAATCTTCAA AAAGACGGGA CAAAGACAGA CAAGTTGGAA 1620

AGGCTAATGG TCAAGATCGG GGTCTTCTCA GTACTGTACA CGGTTCCTGC AACCTGTGTG 1680

ATTGCCTGTT ATTTCTATGA AATCTCAAAC TGGGCACTCT TTCGATATTC TGCAGATGAC 1740

TCAAACATGG CAGTTGAAAT GTTGAAAATT TTTATGTCTT TGCTCGTGGG CATCACTTCA 1800

GGCATGTGGA TTTGGTCTGC CAAAACTCTT CACACGTGGC AAAAGTGTTC TAACCGATTG 1860

GTGAATTCTG GGAAGGTAAA GAGAGAGAAG AGGGGGAATG GTTGGGTGAA GCCAGGAAAA 1920

GGCAACGAGA CTGTGGTATA AGACTAGCCG GCTTCCTCGT TCCTCATTGT GAAGGAAGTG 1980

ATGCAGGGAA TCTCAGTTTG AACAAACTTA GAAACACTTC AGCCCACACA CACCCACGTC 2040

AGCCCACCAC CACTCACCCA ACTCAGCATC AGAAGACCAA TGGCTTCACT GCAGACTTTG 2100

GAATGGTCCA AAATGGAAAA GCCAGTTAAG AGGTTTTCAA AGCTGTGAAA AATCAAAATG 2160

TTGATCACTT TAGCAGGTCA CAGCTTGGAG TCCGTGGAGG TCCCGCCTAG ATTCCTGAAG 2220

CCCAGGGTGA TAGTGTTTGC TCCTACTGGG TGGGATTTCA ACTGTGAGTT GATAACATGC 2280

AAGGAGAAAG ATTAATTTTT AAAACCCTTT TAAATTTTAA ATAGTAACTA AGGTCTTGCA 2340

GATAGCAAAG TGATCTATAA ACACTGGAAA TGCTGGGTTG GGAGACGTGT TGCAGAGTTT 2400

TTATATGTTT CTGGTCTAAC ATAAACATCT TCTGGCCTAC ACTGTCTGCT GTTTAGAACT 2460

CTGTAGCGCA CTCCCAGAGG TGGTGTCAAA ATCCTTCAGT GCCTTGTCGT AAAACAGAAT 2520

TGTTTGAGCA AACAAAAGTA CTGTACTAAC ACACGTAAGG TATCCAGTGG ATTTCTCTCT 2580

CCTGAAATTT CAACATCCCT AATTCTAGGC AGCCCCTGTT TTCTTCACTT TAAACTAATG 2640

ACTCAAAAAA AAAAAGGTTA TTTTTATAGG ATTTTTTTTT GCACTGCAGC ATGCCTAATG 2700

AGAGGAAAAG GAGGTGATCA CTTCTGACAA TCACTTAATT CAGAGAAAAA TGAGATTTGC 2760

TAATTGACTT ACCTTCCGAC CCCTAGAGAC CCTATTGCAT TAAGCAATGT TTAAGCAATT 2820

GGGGACTT 2828

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 538 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Mfz4 protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Ala Trp Pro Gly Thr Gly Pro Ser Ser Arg Gly Ala Pro Gly Gly
1 5 10 15

Val Gly Leu Arg Leu Gly Leu Leu Leu Gln Phe Leu Leu Leu Leu Arg
20 25 30

Pro Thr Leu Gly Phe Gly Asp Glu Glu Glu Arg Arg Cys Asp Pro Ile
35 40 45

Arg Ile Ala Met Cys Gln Asn Leu Gly Tyr Asn Val Thr Lys Met Pro
50 55 60

Asn Leu Val Gly His Glu Leu Gln Thr Asp Ala Glu Leu Gln Leu Thr
65 70 75 80

Thr Phe Thr Pro Leu Ile Gln Tyr Gly Cys Ser Ser Gln Leu Gln Phe
85 90 95

Phe Leu Cys Ser Val Tyr Val Pro Met Cys Thr Glu Lys Ile Asn Ile
100 105 110

Pro Ile Gly Pro Cys Gly Gly Met Cys Leu Ser Val Lys Arg Arg Cys
115 120 125

Glu Pro Val Leu Arg Glu Phe Gly Phe Ala Trp Pro Asp Thr Leu Asn
130 135 140

Cys Ser Lys Phe Pro Pro Gln Asn Asp His Asn His Met Cys Met Glu
145 150 155 160

Gly Pro Gly Asp Glu Glu Val Pro Leu Pro His Lys Thr Pro Ile Gln
165 170 175

Pro Gly Glu Glu Cys His Ser Val Gly Ser Asn Ser Asp Gln Tyr Ile
180 185 190

Trp Val Lys Arg Ser Leu Asn Cys Val Leu Lys Cys Gly Tyr Asp Ala
195 200 205

Gly Leu Tyr Ser Arg Ser Ala Lys Glu Phe Thr Asp Ile Trp Met Ala
210 215 220

Val Trp Ala Ser Leu Cys Phe Ile Ser Thr Thr Phe Thr Val Leu Thr
225 230 235 240

Phe Leu Ile Asp Ser Ser Arg Phe Ser Tyr Pro Glu Arg Pro Ile Ile
245 250 255

Phe Leu Ser Met Cys Tyr Asn Ile Tyr Ser Ile Ala Tyr Ile Val Arg
260 265 270

Leu Thr Val Gly Arg Glu Arg Ile Ser Cys Asp Phe Glu Glu Ala Ala

275 280 285

Glu Pro Val Leu Ile Gln Glu Gly Leu Lys Asn Thr Gly Cys Ala Ile
290 295 300

Ile Phe Leu Leu Met Tyr Phe Phe Gly Met Ala Ser Ser Ile Trp Trp
305 310 315 320

Val Ile Leu Thr Leu Thr Trp Phe Leu Ala Ala Gly Leu Lys Trp Gly
325 330 335

His Glu Ala Ile Glu Met His Ser Ser Tyr Phe His Ile Ala Ala Trp
340 345 350

Ala Ile Pro Ala Val Lys Thr Ile Val Ile Leu Ile Met Arg Leu Val
355 360 365

Asp Ala Asp Glu Leu Thr Gly Leu Cys Tyr Val Gly Asn Gln Asn Leu
370 375 380

Asp Ala Leu Thr Gly Phe Val Val Ala Pro Leu Phe Thr Tyr Leu Val
385 390 395 400

Ile Gly Thr Leu Phe Ile Ala Ala Gly Leu Val Ala Leu Phe Lys Ile
405 410 415

Arg Ser Asn Leu Gln Lys Asp Gly Thr Lys Thr Asp Lys Leu Glu Arg
420 425 430

Leu Met Val Lys Ile Gly Val Phe Ser Val Leu Tyr Thr Val Pro Ala
435 440 445

Thr Cys Val Ile Ala Cys Tyr Phe Tyr Glu Ile Ser Asn Trp Ala Leu
450 455 460

Phe Arg Tyr Ser Ala Asp Asp Ser Asn Met Ala Val Glu Met Leu Lys
465 470 475 480

Ile Phe Met Ser Leu Leu Val Gly Ile Thr Ser Gly Met Trp Ile Trp
485 490 495

Ser Ala Lys Thr Leu His Thr Trp Gln Lys Cys Ser Asn Arg Leu Val
500 505 510

Asn Ser Gly Lys Val Lys Arg Glu Lys Arg Gly Asn Gly Trp Val Lys
515 520 525

Pro Gly Lys Gly Asn Glu Thr Val Val Glx
530 535

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2334 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Human transmembrane receptor (frizzled 5) mRNA, Coding region: 321..2078

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ACCCAGGGAC GGAGGACCCA GGCTGGCTTG GGGACTGTCT GCTCTTCTCG GCGGGAGCCG 60

TGGAGAGTCC TTTCCCTGGA ATCCGAGCCC TAACCGTCTC TCCCCAGCCC TATCCGGCGA 120

GGAGCGGAGC GCTGCCAGCG GAGGCAGCGC CTTCCCGAAG CAGTTTATCT TTGGACGGTT 180

TTCTTTAAAG GAAAAACGAA CCAACAGGTT GCCAGCCCCG GCGCCACACA CGAGACGCCG 240

GAGGGAGAAG CCCCGGCCCG GATTCCTCTG CCTGTGTGCG TCCCTCGCGG GCTGCTGGAG 300

GCGAGGGGAG GGAGGGGGCG ATGGCTCGGC CTGACCCATC CGCGCCGCCC TCGCTGTTGC 360

TGCTGCTCCT GGCGCAGCTG GTGGGCCGGG CGGCCGCCGC GTCCAAGGCC CCGGTGTGCC 420

AGGAAATCAC GGTGCCCATG TGCCGCGGCA TCGGCTACAA CCTGACGCAC ATGCCCAACC 480

AGTTCAACCA CGACACGCAG GACGAGGCGG GCCTGGAGGT GCACCAGTTC TGGCCGCTGG 540

TGGAGATCCA ATGCTCGCCG GACCTGCGCT TCTTCCTATG CACTATGTAC ACGCCCATCT 600

GTCTGCCCGA CTACCACAAG CCGCTGCCGC CCTGCCGCTC GGTGTGCGAG CGCGCCAAGG 660

CCGGCTGCTC GCCGCTGATG CGCCAGTACG GCTTCGCCTG GCCCGAGCGC ATGAGCTGCG 720

ACCGCCTCCC GGTGCTGGGC CGCGACGCCG AGGTCCTCTG CATGGATTAC AACCGCAGCG 780

AGGCCACCAC GGCGCCCCCC AGGCCTTTCC CAGCCAAGCC CACCCTTCCA GGCCCGCCAG 840

GGGCGCCGGC CTCGGGGGGC GAATGCCCCG CTGGGGGCCC GTTCGTGTGC AAGTGTCGCG 900

AGCCCTTCGT GCCCATTCTG AAGGAGTCAC ACCCGCTCTA CAACAAGGTG CGGACGGGCC 960

AGGTGCCCAA CTGCGCGGTA CCCTGCTACC AGCCGTCCTT CAGTGCCGAC GAGCGCACGT 1020

TCGCCACCTT CTGGATAGGC CTGTGGTCGG TGCTGTGCTT CATCTCCACG TCCACCACAG 1080

TGGCCACCTT CCTCATCGAC ATGGACACGT TCCGCTATCC TGAGCGCCCC ATCATCTTCC 1140

TGTCAGCCTG CTACCTGTGC GTGTCGCTGG GCTTCCTGGT GCGTCTGGTC GTGGGCCATG 1200

CCAGCGTGGC CTGCAGCCGC GAGCACAACC ACATCCACTA CGAGACCACG GGCCCTGCAC 1260

TGTGCACCAT CGTCTTCCTC CTGGTCTACT TCTTCGGCAT GGCCAGCTCC ATCTGGTGGG 1320

TCATCCTGTC GCTCACCTGG TTCCTGGCCG CCGCGATGAA GTGGGGCAAC GAGGCCATCG 1380

CGGGCTACGG CCAGTACTTC CACCTGGCTG CGTGGCTCAT CCCCAGCGTC AAGTCCATCA 1440

CGGCACTGGC GCTGAGCTCC GTGGACGGGG ACCCAGTGGC CGGCATCTGC TACGTGGGCA 1500

ACCAGAACCT GAACTCGCTG CGGCGCTTCG TGCTGGGCCC GCTGGTGCTC TACCTGCTGG 1560

TGGGCACGCT CTTCCTGCTG GCGGGCTTCG TGTCGCTCTT CCGCATCCGC AGCGTCATCA 1620

AGCAGGGCGG CACCAAGACG GACAAGCTGG AGAAGCTCAT GATCCGCATC GGCATCTTCA 1680

CGCTGCTCTA CACGGTCCCC GCCAGCATTG TGGTGGCCTG CTACCTGTAC GAGCAGCACT 1740

ACCGCGAGAG CTGGGAGGCG GCGCTCACCT GCGCCTGCCC GGGCCACGAC ACCGGCCAGC 1800

CGCGCGCCAA GCCCGAGTAC TGGGTGCTCA TGCTCAAGTA CTTCATGTGC CTGGTGGTGG 1860

GCATCACGTC GGGCGTCTGG ATCTGGTCGG GCAAGACGGT GGAGTCGTGG CGGCGTTTCA 1920

CCAGCCGCTG CTGCTGCCGC CCGCGGCGCG GCCACAAGAG CGGGGGCGCC ATGGCCGCAG 1980

GGGACTACCC CGAGGCGAGC GCCGCGCTCA CAGGCAGGAC CGGGCCGCCG GGCCCCGCCG 2040

CCACCTACCA CAAGCAGGTG TCCCTGTCGC ACGTGTAGGA GGCTGCCGCC GAGGGACTCG 2100

GCCGGAGAGC TGAGGGGAGG GGGGCGTTTT GTTTGGTAGT TTTGCCAAGG TCACTTCCGT 2160

TTACCTTCAT GGTGCTGTTG CCCCCTCCCG CGGCGACTTG GAGAGAGGGA AGAGGGGCGT 2220

TTTCGAGGAA GAACCTGTCC CAGGTCTTCT CCAAGGGGCC CAGCTCACGT GTATTCTATT 2280

TTGCGTTTCT TACCTGCCTT CTTTATGGGA ACCCTCTTTT TAATTTATAT GTAT 2334

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 586 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Hfz5 protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ala Arg Pro Asp Pro Ser Ala Pro Pro Ser Leu Leu Leu Leu Leu
1 5 10 15

Leu Ala Gln Leu Val Gly Arg Ala Ala Ala Ala Ser Lys Ala Pro Val
20 25 30

Cys Gln Glu Ile Thr Val Pro Met Cys Arg Gly Ile Gly Tyr Asn Leu
35 40 45

Thr His Met Pro Asn Gln Phe Asn His Asp Thr Gln Asp Glu Ala Gly
50 55 60

Leu Glu Val His Gln Phe Trp Pro Leu Val Glu Ile Gln Cys Ser Pro
65 70 75 80

Asp Leu Arg Phe Phe Leu Cys Thr Met Tyr Thr Pro Ile Cys Leu Pro
85 90 95

Asp Tyr His Lys Pro Leu Pro Pro Cys Arg Ser Val Cys Glu Arg Ala
100 105 110

Lys Ala Gly Cys Ser Pro Leu Met Arg Gln Tyr Gly Phe Ala Trp Pro
115 120 125

Glu Arg Met Ser Cys Asp Arg Leu Pro Val Leu Gly Arg Asp Ala Glu
130 135 140

Val Leu Cys Met Asp Tyr Asn Arg Ser Glu Ala Thr Thr Ala Pro Pro
145 150 155 160

Arg Pro Phe Pro Ala Lys Pro Thr Leu Pro Gly Pro Pro Gly Ala Pro
165 170 175

Ala Ser Gly Gly Glu Cys Pro Ala Gly Gly Pro Phe Val Cys Lys Cys
180 185 190

Arg Glu Pro Phe Val Pro Ile Leu Lys Glu Ser His Pro Leu Tyr Asn
195 200 205

Lys Val Arg Thr Gly Gln Val Pro Asn Cys Ala Val Pro Cys Tyr Gln
210 215 220

Pro Ser Phe Ser Ala Asp Glu Arg Thr Phe Ala Thr Phe Trp Ile Gly
225 230 235 240

Leu Trp Ser Val Leu Cys Phe Ile Ser Thr Ser Thr Thr Val Ala Thr
245 250 255

Phe Leu Ile Asp Met Asp Thr Phe Arg Tyr Pro Glu Arg Pro Ile Ile
260 265 270

Phe Leu Ser Ala Cys Tyr Leu Cys Val Ser Leu Gly Phe Leu Val Arg
275 280 285

Leu Val Val Gly His Ala Ser Val Ala Cys Ser Arg Glu His Asn His
290 295 300

Ile His Tyr Glu Thr Thr Gly Pro Ala Leu Cys Thr Ile Val Phe Leu
305 310 315 320

Leu Val Tyr Phe Phe Gly Met Ala Ser Ser Ile Trp Trp Val Ile Leu
325 330 335

Ser Leu Thr Trp Phe Leu Ala Ala Ala Met Lys Trp Gly Asn Glu Ala
340 345 350

Ile Ala Gly Tyr Gly Gln Tyr Phe His Leu Ala Ala Trp Leu Ile Pro
355 360 365

Ser Val Lys Ser Ile Thr Ala Leu Ala Leu Ser Ser Val Asp Gly Asp
370 375 380

Pro Val Ala Gly Ile Cys Tyr Val Gly Asn Gln Asn Leu Asn Ser Leu
385 390 395 400

Arg Arg Phe Val Leu Gly Pro Leu Val Leu Tyr Leu Leu Val Gly Thr
405 410 415

Leu Phe Leu Leu Ala Gly Phe Val Ser Leu Phe Arg Ile Arg Ser Val
420 425 430

Ile Lys Gln Gly Gly Thr Lys Thr Asp Lys Leu Glu Lys Leu Met Ile
435 440 445

Arg Ile Gly Ile Phe Thr Leu Leu Tyr Thr Val Pro Ala Ser Ile Val
450 455 460

Val Ala Cys Tyr Leu Tyr Glu Gln His Tyr Arg Glu Ser Trp Glu Ala
465 470 475 480

Ala Leu Thr Cys Ala Cys Pro Gly His Asp Thr Gly Gln Pro Arg Ala
485 490 495

Lys Pro Glu Tyr Trp Val Leu Met Leu Lys Tyr Phe Met Cys Leu Val
500 505 510

Val Gly Ile Thr Ser Gly Val Trp Ile Trp Ser Gly Lys Thr Val Glu
515 520 525

Ser Trp Arg Arg Phe Thr Ser Arg Cys Cys Cys Arg Pro Arg Arg Gly

530 535 540

His Lys Ser Gly Gly Ala Met Ala Ala Gly Asp Tyr Pro Glu Ala Ser
545 550 555 560

Ala Ala Leu Thr Gly Arg Thr Gly Pro Pro Gly Pro Ala Ala Thr Tyr
565 570 575

His Lys Gln Val Ser Leu Ser His Val Glx
580 585

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2492 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Mus musculus putative transmembrane receptor (frizzled 6) mRNA, Coding region: 146..2275

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TCATTTCAGG CCCAGCTACT ATCAAAATGG TACAAAGAAT GCAATGAGGA ATTTGTACAT 60

TTTATCTCTG ATTTGAGAAT CTTTTTGATG CGGAAAGGAG CATAAGAATA ATCCAAGCCA 120

TGTGGTAAAA TCGGAGTCTG GCAAGATGGA AAGGTCCCCG TTTCTGTTGG CGTGCATTCT 180

TCTGCCCCTC GTAAGAGGAC ACAGCCTTTT CACCTGTGAG CCAATCACCG TTCCCAGATG 240

TATGAAAATG ACTTACAACA TGACGTTCTT CCCTAACCTG ATGGGTCATT ATGACCAGGG 300

GATCGCTGCT GTGGAAATGG GGCACTTTCT GCATCTTGCA AATCTAGAAT GTTCACCAAA 360

CATTGAAATG TTCCTTTGCC AAGCTTTTAT ACCAACCTGC ACAGAGCAAA TTCATGTAGT 420

TCTACCCTGT CGGAAATTGT GTGAGAAAAT AGTTTCTGAT TGCAAAAAAC TAATGGACAC 480

TTTTGGCATC CGATGGCCTG AAGAACTTGA ATGTAACAGA TTGCCACACT GTGATGACAC 540

TGTTCCTGTA ACTTCTCATC CACACACAGA GCTTTCTGGG CCACAGAAGA AATCAGATCA 600

AGTCCCAAGA GACATTGGAT TTTGGTGTCC AAAGCACCTT AGGACTTCCG GGGACCAAGG 660

CTATAGGTTT CTGGGAATTG AACAGTGTGC CCCTCCGTGC CCCAATATGT ATTTTAAAAG 720

TGATGAACTA GACTTTGCCA AAAGTTTCAT AGGAATAGTT TCAATATTTT GTCTTTGTGC 780

AACTCTGTTC ACGTTCCTTA CATTTTTAAT TGACGTTAGA CGATTCAGAT ACCCAGAGAG 840

ACCAATTATC TATTACTCTG TCTGCTACAG CATTGTCTCT CTCATGTACT TCGTGGGGTT 900

TTTGCTGGGC AATAGCACAG CTTGTAATAA GGCAGACGAG AAGCTGGAGC TCGGGGACAC 960

CGTTGTCCTA GGGTCAAAGA ATAAGGCTTG CAGTGTGGTA TTTATGTTTC TGTATTTTTT 1020

TACAATGGCT GGCACCGTGT GGTGGGTGAT TCTCACCATT ACGTGGTTCT TAGCTGCCGG 1080

GAGAAAATGG AGTTGCGAAG CTATTGAACA AAAAGCAGTG TGGTTCCATG CCGTTGCCTG 1140

GGGGGCGCCC GGGTTCCTGA CCGTCATGCT GCTCGCTATG AATAAGGTTG AAGGAGACAA 1200

CATTAGCGGC GTTTGCTTCG TTGGCCTGTA TGACCTGGAC GCCTCTCGCT ACTTCGTCCT 1260

TCTGCCTCTG TGCCTCTGCG TATTTGTTGG GCTGTCTCTC CTCTTAGCCG GCATCATCTC 1320

CTTGAATCAT GTCCGACAAG TCATACAGCA TGATGGTCGG AACCAAGAGA AGCTAAAGAA 1380

ATTCATGATT CGCATCGGAG TCTTCAGTGG CCTGTATCTT GTGCCCTTAG TGACACTTCT 1440

CGGTTGCTAT GTCTATGAGC TAGTGAACAG GATCACCTGG GAGATGACAT GGTTCTCTGA 1500

TCATTGTCAC CAGTACCGCA TCCCGTGCCC TTACCAGGCA AATCCAAAAG CTCGACCAGA 1560

ATTGGCTTTA TTTATGATAA AATATCTGAT GACATTAATT GTTGGTATCT CTGCGGTCTT 1620

CTGGGTTGGA AGCAAAAAGA CGTGCACAGA ATGGGCCGGG TTCTTTAAGC GAAACCGCAA 1680

GCGAGACCCC ATCAGTGAGA GCCGCCGAGT GCTGCAAGAG TCCTGTGAGT TCTTCCTGAA 1740

GCACAACTCT AAAGTGAAGC ACAAGAAGAA GCATGGCGCA CCAGGGCCTC ATAGGCTGAA 1800

GGTCATTTCC AAGTCCATGG GAACTAGCAC AGGAGCGACC ACAAATCATG GCACCTCTGC 1860

CATGGCAATC GCTGACCATG ATTACTTAGG GCAAGAAACT TCAACAGAAG TCCACACCTC 1920

CCCAGAAGCA TCCGTCAAAG AGGGACGAGC AGACCGAGCA AACACTCCCA GCGCCAAAGA 1980

TCGGGACTGT GGGGAATCTG CAGGGCCCAG TTCCAAGCTC TCTGGGAACC GGAACGGCAG 2040

GGAAAGCCGA GCGGGCGGCC TGAAGGAGAG AAGCAATGGA TCAGAGGGGG CTCCAAGTGA 2100

AGGAAGGGTA AGTCCAAAGA GCAGCGTTCC TGAGACTGGC CTGATAGACT GCAGCACTTC 2160

ACAGGCCGCC AGTTCTCCAG AACCAACCAG CCTCAAGGGC TCCACATCTC TGCCTGTTCA 2220

CTCAGCTTCC AGAGCTAGGA AAGAGCAGGG TGCTGGCAGC CATTCCGACG CTTGAAGAAA 2280

ACTGTCTCGT TCCCCCAGAA GCACATGTAT GTTACACTGG AGATGACCAA CTGATTTGTC 2340

TTATAAAGGC CACTGTTGAG CTGGGAGAGT AGCCCAGTGG TACAGCGCCC ACCTGGAATA 2400

CTGAGGACCT GGGGTTGTCT CCCAGCACTG CAAAAGGAAA ATTCACTGTT ACAGTCTTCC 2460

TTGCACTTAA CCAGCTTTGT CTATGTTTTT TT 2492

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 710 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Mfz6 protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Glu Arg Ser Pro Phe Leu Leu Ala Cys Ile Leu Leu Pro Leu Val
1 5 10 15

Arg Gly His Ser Leu Phe Thr Cys Glu Pro Ile Thr Val Pro Arg Cys
20 25 30

Met Lys Met Thr Tyr Asn Met Thr Phe Phe Pro Asn Leu Met Gly His
35 40 45

Tyr Asp Gln Gly Ile Ala Ala Val Glu Met Gly His Phe Leu His Leu
50 55 60

Ala Asn Leu Glu Cys Ser Pro Asn Ile Glu Met Phe Leu Cys Gln Ala
65 70 75 80

Phe Ile Pro Thr Cys Thr Glu Gln Ile His Val Val Leu Pro Cys Arg
85 90 95

Lys Leu Cys Glu Lys Ile Val Ser Asp Cys Lys Lys Leu Met Asp Thr
100 105 110

Phe Gly Ile Arg Trp Pro Glu Glu Leu Glu Cys Asn Arg Leu Pro His
115 120 125

Cys Asp Asp Thr Val Pro Val Thr Ser His Pro His Thr Glu Leu Ser
130 135 140

Gly Pro Gln Lys Lys Ser Asp Gln Val Pro Arg Asp Ile Gly Phe Trp
145 150 155 160

Cys Pro Lys His Leu Arg Thr Ser Gly Asp Gln Gly Tyr Arg Phe Leu
165 170 175

Gly Ile Glu Gln Cys Ala Pro Pro Cys Pro Asn Met Tyr Phe Lys Ser
180 185 190

Asp Glu Leu Asp Phe Ala Lys Ser Phe Ile Gly Ile Val Ser Ile Phe
195 200 205

Cys Leu Cys Ala Thr Leu Phe Thr Phe Leu Thr Phe Leu Ile Asp Val
210 215 220

Arg Arg Phe Arg Tyr Pro Glu Arg Pro Ile Ile Tyr Tyr Ser Val Cys
225 230 235 240

Tyr Ser Ile Val Ser Leu Met Tyr Phe Val Gly Phe Leu Leu Gly Asn
245 250 255

Ser Thr Ala Cys Asn Lys Ala Asp Glu Lys Leu Glu Leu Gly Asp Thr
260 265 270

Val Val Leu Gly Ser Lys Asn Lys Ala Cys Ser Val Val Phe Met Phe
275 280 285

Leu Tyr Phe Phe Thr Met Ala Gly Thr Val Trp Trp Val Ile Leu Thr
290 295 300

Ile Thr Trp Phe Leu Ala Ala Gly Arg Lys Trp Ser Cys Glu Ala Ile
305 310 315 320

Glu Gln Lys Ala Val Trp Phe His Ala Val Ala Trp Gly Ala Pro Gly
325 330 335

Phe Leu Thr Val Met Leu Leu Ala Met Asn Lys Val Glu Gly Asp Asn
340 345 350

Ile Ser Gly Val Cys Phe Val Gly Leu Tyr Asp Leu Asp Ala Ser Arg

355 360 365

Tyr Phe Val Leu Leu Pro Leu Cys Leu Cys Val Phe Val Gly Leu Ser
370 375 380

Leu Leu Leu Ala Gly Ile Ile Ser Leu Asn His Val Arg Gln Val Ile
385 390 395 400

Gln His Asp Gly Arg Asn Gln Glu Lys Leu Lys Lys Phe Met Ile Arg
405 410 415

Ile Gly Val Phe Ser Gly Leu Tyr Leu Val Pro Leu Val Thr Leu Leu
420 425 430

Gly Cys Tyr Val Tyr Glu Leu Val Asn Arg Ile Thr Trp Glu Met Thr
435 440 445

Trp Phe Ser Asp His Cys His Gln Tyr Arg Ile Pro Cys Pro Tyr Gln
450 455 460

Ala Asn Pro Lys Ala Arg Pro Glu Leu Ala Leu Phe Met Ile Lys Tyr
465 470 475 480

Leu Met Thr Leu Ile Val Gly Ile Ser Ala Val Phe Trp Val Gly Ser
485 490 495

Lys Lys Thr Cys Thr Glu Trp Ala Gly Phe Phe Lys Arg Asn Arg Lys
500 505 510

Arg Asp Pro Ile Ser Glu Ser Arg Arg Val Leu Gln Glu Ser Cys Glu
515 520 525

Phe Phe Leu Lys His Asn Ser Lys Val Lys His Lys Lys Lys His Gly
530 535 540

Ala Pro Gly Pro His Arg Leu Lys Val Ile Ser Lys Ser Met Gly Thr
545 550 555 560

Ser Thr Gly Ala Thr Thr Asn His Gly Thr Ser Ala Met Ala Ile Ala
565 570 575

Asp His Asp Tyr Leu Gly Gln Glu Thr Ser Thr Glu Val His Thr Ser
580 585 590

Pro Glu Ala Ser Val Lys Glu Gly Arg Ala Asp Arg Ala Asn Thr Pro
595 600 605

Ser Ala Lys Asp Arg Asp Cys Gly Glu Ser Ala Gly Pro Ser Ser Lys
610 615 620

Leu Ser Gly Asn Arg Asn Gly Arg Glu Ser Arg Ala Gly Gly Leu Lys
625 630 635 640

Glu Arg Ser Asn Gly Ser Glu Gly Ala Pro Ser Glu Gly Arg Val Ser
645 650 655

Pro Lys Ser Ser Val Pro Glu Thr Gly Leu Ile Asp Cys Ser Thr Ser
660 665 670

Gln Ala Ala Ser Ser Pro Glu Pro Thr Ser Leu Lys Gly Ser Thr Ser
675 680 685

Leu Pro Val His Ser Ala Ser Arg Ala Arg Lys Glu Gln Gly Ala Gly
690 695 700

Ser His Ser Asp Ala Glx
705 710

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2259 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Mus musculus transmembrane receptor (frizzled 7) mRNA, Coding region: 362..2080

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TTTGAAGGTA ACCGGAGAAG CTTGTTGCTC GTCGCCGCAG AGAAAGCCGC ACCGTTACGT 60

CTCGGGGGGA GGGTAAGGCG ACACCCCTTC CCTCGTACCC CCACTCCAGG CCCAGGAGTT 120

TGAACTCCGG CGGCTGCGTG AGTGCCACGT GGAGGCGGCT GCGGCGCCCC TCGGCTGGCG 180

GCCTCGCCCC CGCTGTGCAG GCACCCTAGC ACCCTCGGCT CCGCGCCGCC CACGGCGGCC 240

CCGGCGCCGG GAGGACTCTC ATGCGCCGGC CGGGCGGCGG CGCCTCCCTG TATCCAAGCC 300

TCTCCCCAGC GCCTCGTCTT TTTCCTCCAG CTGAGAACGC CGCTGCACTC GCGACCGGCG 360

ATGCGGGGCC CCGGCACGGC GGCGTCGCAC TCGCCCCTGG GCCTCTGCGC CCTGGTGCTT 420

GCTCTTCTGG GCGCGCTGCC CACGGACACC CGGGCTCAGC CATATCACGG CGAGAAAGGC 480

ATCTCGGTAC CGGACCACGG CTTCTGCCAG CCCATCTCCA TCCCGTTGTG CACGGATATC 540

GCCTACAACC AGACCATCCT GCCCAACCTG CTGGGCCACA CGAACCAAGA GGACGCGGGC 600

CTCGAGGTGC ACCAGTTCTA CCCTCTGGTA AAGGTGCAGT GTTCTCCTGA GCTACGCTTC 660

TTCTTATGCT CTATGTACGC ACCCGTGTGC ACCGTGCTCG ACCAAGCCAT TCCTCCGTGC 720

CGTTCCTTGT GCGAGCGCGC CCGACAGGGC TGCGAGGCGC TCATGAACAA GTTCGGCTTC 780

CAGTGGCCAG AGCGGTTGCG CTGCGAGAAC TTCCCAGTGC ACGGTGCCGG CGAGATCTGC 840

GTGGGGCAGA ACACGTCCGA CGGCTCCGGG GGCGCGGGCG GCAGTCCCAC CGCCTACCCT 900

ACTGCTCCCT ACCTGCCAGA CCCACCTTTC ACTGCGATGT CCCCCTCAGA TGGCAGAGGC 960

CGCTTGTCTT TCCCCTTCTC GTGTCCGCGC CAGCTCAAGG TGCCCCCCTA CCTGGGCTAC 1020

CGCTTCCTAG GTGAGCGTGA CTGCGGTGCC CCGTGTGAGC CGGGCCGTGC TAACGGCCTC 1080

ATGTACTTTA AAGAAGAGGA GAGACGGTTC GCCCGCCTCT GGGTGGGTGT GTGGTCAGTG 1140

CTGTCGTGCG CCTCGACGCT CTTCACGGTG CTCACCTACC TAGTGGACAT GCGTCGCTTC 1200

AGCTATCCAG AGCGACCCAT CATCTTCCTG TCGGGTTGCT ACTTCATGGT GGCAGTGGCG 1260

CACGTGGCAG GCTTCCTGCT AGAGGACCGT GCCGTGTGCG TGGAGCGCTT CTCGGACGAT 1320

GGCTACCGCA CGGTGGCGCA GGGCACCAAG AAGGAGGGCT GCACCATCCT CTTCATGGTG 1380

CTTTACTTCT TCGGTATGGC CAGCTCCATC TGGTGGGTCA TTCTGTCCCT CACTTGGTTC 1440

CTGGCAGCTG GCATGAAGTG GGGCCACGAG GCCATCGAGG CCAACTCGCA GTACTTTCAT 1500

CTGGCCGCGT GGGCTGTGCC AGCGGTCAAG ACAATCACCA TTTTGGCCAT GGGCCAGGTG 1560

GATGGTGACC TACTCAGTGG AGTGTGCTAC GTGGGCCTGT CTAGTGTGGA TGCATTGCGG 1620

GGCTTCGTGC TGGCGCCCTT GTTCGTCTAC CTCTTCATCG GGACGTCCTT CCTGTTGGCC 1680

GGCTTTGTGT CTCTCTTTCG CATCCGCACC ATCATGAAGC ACGACGGCAC CAAGACAGAG 1740

AAGCTGGAGA AGCTGATGGT GCGCATCGGC GTCTTCAGCG TGCTCTACAC GGTGCCGGCC 1800

ACCATCGTGT TGGCCTGCTA CTTTTATGAG CAGGCCTTCC GAGAGCACTG GGAACGCACC 1860

TGGCTCCTGC AGACTTGCAA GAGCTACGCT GTGCCCTGCC CTCCGCGCCA CTTCTCTCCC 1920

ATGAGCCCCG ACTTTACAGT CTTCATGATC AAGTACCTGA TGACCATGAT CGTGGGCATC 1980

ACTACGGGCT TCTGGATCTG GTCGGGCAAG ACCCTGCAGT CATGGCGTCG CTTCTACCAC 2040

AGACTCAGCC ACAGCAGCAA GGGGGAAACT GCGGTATGAG CCCCGGTCCT TACCCACCCT 2100

TGCCTCTTCT ACCCTTTTAC AGGAGGAGAG GCATGGTAGG GAGAGAACTG CTGGGTGGGG 2160

GCTTGTTTCC GTAAGCTACC TGCCCCCTCC ACTGAGCTTT AACCTGGAAG TGAGAAGTTA 2220

TTTGGAGGTG AGAAGAGATT TGGGGGCGAG AGATGGTTT 2259

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 573 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Mfz7 protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Arg Gly Pro Gly Thr Ala Ala Ser His Ser Pro Leu Gly Leu Cys
1 5 10 15

Ala Leu Val Leu Ala Leu Leu Gly Ala Leu Pro Thr Asp Thr Arg Ala
20 25 30

Gln Pro Tyr His Gly Glu Lys Gly Ile Ser Val Pro Asp His Gly Phe
35 40 45

Cys Gln Pro Ile Ser Ile Pro Leu Cys Thr Asp Ile Ala Tyr Asn Gln
50 55 60

Thr Ile Leu Pro Asn Leu Leu Gly His Thr Asn Gln Glu Asp Ala Gly
65 70 75 80

Leu Glu Val His Gln Phe Tyr Pro Leu Val Lys Val Gln Cys Ser Pro
85 90 95

Glu Leu Arg Phe Phe Leu Cys Ser Met Tyr Ala Pro Val Cys Thr Val
100 105 110
Leu Asp Gln Ala Ile Pro Pro Cys Arg Ser Leu Cys Glu Arg Ala Arg
115 120 125
Gln Gly Cys Glu Ala Leu Met Asn Lys Phe Gly Phe Gln Trp Pro Glu
130 135 140
Arg Leu Arg Cys Glu Asn Phe Pro Val His Gly Ala Gly Glu Ile Cys
145 150 155 160
Val Gly Gln Asn Thr Ser Asp Gly Ser Gly Gly Ala Gly Gly Ser Pro
165 170 175
Thr Ala Tyr Pro Thr Ala Pro Tyr Leu Pro Asp Pro Pro Phe Thr Ala
180 185 190
Met Ser Pro Ser Asp Gly Arg Gly Arg Leu Ser Phe Pro Phe Ser Cys
195 200 205
Pro Arg Gln Leu Lys Val Pro Pro Tyr Leu Gly Tyr Arg Phe Leu Gly
210 215 220
Glu Arg Asp Cys Gly Ala Pro Cys Glu Pro Gly Arg Ala Asn Gly Leu
225 230 235 240
Met Tyr Phe Lys Glu Glu Glu Arg Arg Phe Ala Arg Leu Trp Val Gly
245 250 255
Val Trp Ser Val Leu Ser Cys Ala Ser Thr Leu Phe Thr Val Leu Thr
260 265 270
Tyr Leu Val Asp Met Arg Arg Phe Ser Tyr Pro Glu Arg Pro Ile Ile
275 280 285
Phe Leu Ser Gly Cys Tyr Phe Met Val Ala Val Ala His Val Ala Gly
290 295 300
Phe Leu Leu Glu Asp Arg Ala Val Cys Val Glu Arg Phe Ser Asp Asp
305 310 315 320
Gly Tyr Arg Thr Val Ala Gln Gly Thr Lys Lys Glu Gly Cys Thr Ile
325 330 335
Leu Phe Met Val Leu Tyr Phe Phe Gly Met Ala Ser Ser Ile Trp Trp
340 345 350
Val Ile Leu Ser Leu Thr Trp Phe Leu Ala Ala Gly Met Lys Trp Gly
355 360 365
His Glu Ala Ile Glu Ala Asn Ser Gln Tyr Phe His Leu Ala Ala Trp
370 375 380
Ala Val Pro Ala Val Lys Thr Ile Thr Ile Leu Ala Met Gly Gln Val
385 390 395 400
Asp Gly Asp Leu Leu Ser Gly Val Cys Tyr Val Gly Leu Ser Ser Val
405 410 415
Asp Ala Leu Arg Gly Phe Val Leu Ala Pro Leu Phe Val Tyr Leu Phe
420 425 430
Ile Gly Thr Ser Phe Leu Leu Ala Gly Phe Val Ser Leu Phe Arg Ile
435 440 445
Arg Thr Ile Met Lys His Asp Gly Thr Lys Thr Glu Lys Leu Glu Lys

450 455 460

Leu Met Val Arg Ile Gly Val Phe Ser Val Leu Tyr Thr Val Pro Ala
465 470 475 480

Thr Ile Val Leu Ala Cys Tyr Phe Tyr Glu Gln Ala Phe Arg Glu His
485 490 495

Trp Glu Arg Thr Trp Leu Leu Gln Thr Cys Lys Ser Tyr Ala Val Pro
500 505 510

Cys Pro Pro Arg His Phe Ser Pro Met Ser Pro Asp Phe Thr Val Phe
515 520 525

Met Ile Lys Tyr Leu Met Thr Met Ile Val Gly Ile Thr Thr Gly Phe
530 535 540

Trp Ile Trp Ser Gly Lys Thr Leu Gln Ser Trp Arg Arg Phe Tyr His
545 550 555 560

Arg Leu Ser His Ser Ser Lys Gly Glu Thr Ala Val Glx
565 570

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2421 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Mus musculus transmembrane receptor (frizzled 8) gene, Coding region: 188..2245

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGGGGAGGGC CGGACGACTC CAGCCTAGGT TTCCAACCCT GCTGCCTGAA AAGGAGATAG 60

ACTGTTGCTA TTCTCCTCTG CAGAGAAAAG TGGGACACGA CCCGCTCTCC CTTTTCTCAG 120

ATTCCTCACT GCAGAGCCCT CCTGCGCGCC GCCTAGAGAA GGAGGACTTG GGGTCCCAGC 180

GCGCAGCATG GAGTGGGGTT ACCTGTTGGA AGTGACCTCG CTCCTAGCCG CCTTGGCGGT 240

GCTACAGCGC TCTAGCGGCG CTGCCGCGGC TTCGGCCAAG GAGCTGGCGT GCCAAGAGAT 300

CACGGTGCCG TTGTGCAAAG GCATCGGTTA CAACTACACT TACATGCCCA ACCAGTTCAA 360

CCACGACACG CAAGATGAGG CGGGCCTAGA GGTGCACCAG TTTTGGCCGC TGGTGGAGAT 420

ACAGTGCTCC CCGGACCTCA AGTTCTTTCT GTGTAGCATG TACACGCCCA TCTGCCTGGA 480

GGACTACAAG AAGCCTCTGC CGCCTTGTCG CTCTGTGTGT GAACGCGCCA AGGCCGGCTG 540

CGCGCCGCTC ATGCGCCAGT ACGGCTTTGC TTGGCCTGAC CGCATGCGCT GCGATCGGTT 600

GCCGGAGCAG GGCAACCCGG ACACTCTGTG CATGGACTAC AACCGCACCG ACCTCACCAC 660

GGCCGCGCCC AGCCCACCGC GCCGCCTGCC TCCGCCGCCT CCTCCCGGCG AGCAGCCGCC 720

CTCTGGCAGC GGCCACAGCC GCCCGCCAGG GGCCAGGCCC CCACATCGTG GCGGCAGCAG 780

TAGGGGCAGC GGGGACGCGG CGGCTGCGCC CCCTTCGCGC GGCGGGAAGG CGAGGCCCCC 840

TGGTGGCGGC GCTGCTCCCT GCGAGCCGGG GTGCCAGTGC CGCGCGCCCA TGGTGAGCGT 900

GTCCAGCGAA CGCCACCCGC TCTACAACCG CGTCAAGACC GGCCAGATCG CCAACTGTGC 960

GCTGCCCTGC CACAACCCCT TCTTTAGCCA GGATGAGCGC GCCTTCACCG TCTTCTGGAT 1020

CGGCCTGTGG TCGGTGCTCT GCTTCGTCTC CACCTTCGCC ACTGTCTCTA CCTTCCTCAT 1080

CGATATGGAG CGCTTTAAGT ACCCGGAACG GCCCATCATA TTCCTCTCCG CCTGTTACCT 1140

CTTCGTGTCT GTCGGGTACC TGGTGCGCCT GGTGGCAGGA CATGAGAAAG TGGCCTGCAG 1200

CGGCGGCGCT CCGGGTGCTG GCGGACGTGG GGGTGCGGGC GGCGCGGCGG CGGCTGGCGC 1260

AGGGGCAGCG GGACGGGGGG CGAGCAGCCC GGGCGCGCGC GGCGAGTACG AGGAGCTGGG 1320

CGCAGTTGAG CAGCATGTTC GCTATGAGAC CACTGGCCCC GCGCTGTGCA CGGTGGTCTT 1380

TCTCCTTGTC TACTTTTTTG GCATGGCCAG CTCCATCTGG TGGGTAATCC TGTCGCTCAC 1440

GTGGTTCTTG GCAGCTGGCA TGAAGTGGGG TAACGAGGCC ATAGCAGGCT ACTCGCAGTA 1500

CTTCCACCTG GCCGCGTGGC TTGTGCCCAG CGTCAAGTCC ATCGCGGTGC TGGCGCTCAG 1560

CTCCGTAGAC GGCGACCCGG TGGCGGGCAT CTGCTACGTG GGCAACCAGA GCCTTGACAA 1620

CCTACGCGGC TTTGTGCTGG CGCCACTGGT TATCTACCTC TTCATTGGGA CTATGTTTCT 1680

GTTAGCTGGC TTCGTGTCGC TGTTCCGAAT CCGTTCAGTC ATCAAGCAGC AAGGAGGTCC 1740

AACTAAGACA CACAAGCTAG AAAAACTCAT GATCCGCTTG GGCCTCTTCA CCGTGCTCTA 1800

CACGGTGCCC GCTGCCGTCG TTGTCGCCTG CCTTTTCTAT GAGCAGCACA ACCGACCGCG 1860

CTGGGAGGCC ACGCACAACT GCCCATGCCT TCGGGACCTG CAACCGGACC AGGCTCGCAG 1920

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GGGCGTGTGG GTCTGGTCCG GCAAGACTCT GGAGTCCTGG CGCGCGTTGT GCACTAGGTG 2040

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CAGTGGACCC GGGCCCGGCG GAGGTGGGGG ACACGGCGGA GGCGGGGGAT CCCTCTACAG 2160

CGACGTCAGT ACCGGCCTGA CGTGGCGGTC TGGCACGGCC AGCTCTGTAT CTTACCCTAA 2220

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TGGGGGATGG GGAACCCGTG GGCGGCGAAG GGACCCCAGA CCGGCCAGGG TTCCCACCCC 2340

TTCCCAGTGT TGACTGCTAT AGCATGACAA TGAAGTGTTA ATGGTATCCA TTAGCAGCGG 2400

GGACTTAAAT GACTCCCTTA G 2421

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 682 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Mfz8 protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Glu Trp Gly Tyr Leu Leu Glu Val Thr Ser Leu Leu Ala Ala Leu
1 5 10 15

Ala Val Leu Gln Arg Ser Ser Gly Ala Ala Ala Ala Ser Ala Lys Glu
20 25 30

Leu Ala Cys Gln Glu Ile Thr Val Pro Leu Cys Lys Gly Ile Gly Tyr
35 40 45

Asn Tyr Thr Tyr Met Pro Asn Gln Phe Asn His Asp Thr Gln Asp Glu
50 55 60

Ala Gly Leu Glu Val His Gln Phe Trp Pro Leu Val Glu Ile Gln Cys
65 70 75 80

Ser Pro Asp Leu Lys Phe Phe Leu Cys Ser Met Tyr Thr Pro Ile Cys
85 90 95

Leu Glu Asp Tyr Lys Lys Pro Leu Pro Pro Cys Arg Ser Val Cys Glu
100 105 110

Arg Ala Lys Ala Gly Cys Ala Pro Leu Met Arg Gln Tyr Gly Phe Ala
115 120 125

Trp Pro Asp Arg Met Arg Cys Asp Arg Leu Pro Glu Gln Gly Asn Pro
130 135 140

Asp Thr Leu Cys Met Asp Tyr Asn Arg Thr Asp Leu Thr Thr Ala Ala
145 150 155 160

Pro Ser Pro Pro Arg Arg Leu Pro Pro Pro Pro Pro Pro Gly Glu Gln
165 170 175

Pro Pro Ser Gly Ser Gly His Ser Arg Pro Pro Gly Ala Arg Pro Pro
180 185 190

His Arg Gly Gly Ser Ser Arg Gly Ser Gly Asp Ala Ala Ala Ala Pro
195 200 205

Pro Ser Arg Gly Gly Lys Ala Arg Pro Pro Gly Gly Gly Ala Ala Pro
210 215 220

Cys Glu Pro Gly Cys Gln Cys Arg Ala Pro Met Val Ser Val Ser Ser
225 230 235 240

Glu Arg His Pro Leu Tyr Asn Arg Val Lys Thr Gly Gln Ile Ala Asn
245 250 255

Cys Ala Leu Pro Cys His Asn Pro Phe Phe Ser Gln Asp Glu Arg Ala
260 265 270

Phe Thr Val Phe Trp Ile Gly Leu Trp Ser Val Leu Cys Phe Val Ser
275 280 285

Thr Phe Ala Thr Val Ser Thr Phe Leu Ile Asp Met Glu Arg Phe Lys
290 295 300

Tyr Pro Glu Arg Pro Ile Ile Phe Leu Ser Ala Cys Tyr Leu Phe Val

305 310 315 320
Ser Val Gly Tyr Leu Val Arg Leu Val Ala Gly His Glu Lys Val Ala
325 330 335
Cys Ser Gly Gly Ala Pro Gly Ala Gly Gly Arg Gly Gly Ala Gly Gly
340 345 350
Ala Ala Ala Ala Gly Ala Gly Ala Ala Gly Arg Gly Ala Ser Ser Pro
355 360 365
Gly Ala Arg Gly Glu Tyr Glu Glu Leu Gly Ala Val Glu Gln His Val
370 375 380
Arg Tyr Glu Thr Thr Gly Pro Ala Leu Cys Thr Val Val Phe Leu Leu
385 390 395 400
Val Tyr Phe Phe Gly Met Ala Ser Ser Ile Trp Trp Val Ile Leu Ser
405 410 415
Leu Thr Trp Phe Leu Ala Ala Gly Met Lys Trp Gly Asn Glu Ala Ile
420 425 430
Ala Gly Tyr Ser Gln Tyr Phe His Leu Ala Ala Trp Leu Val Pro Ser
435 440 445
Val Lys Ser Ile Ala Val Leu Ala Leu Ser Ser Val Asp Gly Asp Pro
450 455 460
Val Ala Gly Ile Cys Tyr Val Gly Asn Gln Ser Leu Asp Asn Leu Arg
465 470 475 480
Gly Phe Val Leu Ala Pro Leu Val Ile Tyr Leu Phe Ile Gly Thr Met
485 490 495
Phe Leu Leu Ala Gly Phe Val Ser Leu Phe Arg Ile Arg Ser Val Ile
500 505 510
Lys Gln Gln Gly Gly Pro Thr Lys Thr His Lys Leu Glu Lys Leu Met
515 520 525
Ile Arg Leu Gly Leu Phe Thr Val Leu Tyr Thr Val Pro Ala Ala Val
530 535 540
Val Val Ala Cys Leu Phe Tyr Glu Gln His Asn Arg Pro Arg Trp Glu
545 550 555 560
Ala Thr His Asn Cys Pro Cys Leu Arg Asp Leu Gln Pro Asp Gln Ala
565 570 575
Arg Arg Pro Asp Tyr Ala Val Phe Met Leu Lys Tyr Phe Met Cys Leu
580 585 590
Val Val Gly Ile Thr Ser Gly Val Trp Val Trp Ser Gly Lys Thr Leu
595 600 605
Glu Ser Trp Arg Ala Leu Cys Thr Arg Cys Cys Trp Ala Ser Lys Gly
610 615 620
Ala Ala Val Gly Ala Gly Ala Gly Gly Ser Gly Pro Gly Gly Ser Gly
625 630 635 640
Pro Gly Pro Gly Gly Gly Gly Gly His Gly Gly Gly Gly Gly Ser Leu
645 650 655
Tyr Ser Asp Val Ser Thr Gly Leu Thr Trp Arg Ser Gly Thr Ala Ser
660 665 670

Ser Val Ser Tyr Pro Lys Gln Met Pro Leu
675 680

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Amino acid sequence used to design YW157 sense primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Tyr Pro Glu Arg Pro Ile
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Amino acid sequence used to design YW158 antisense primer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Trp Phe Leu Ala Ala
1 5

IT IS CLAIMED:

1. A method of identifying a compound capable of affecting binding of a Wnt polypeptide to a Wnt receptor (WntR) polypeptide, comprising

contacting such a WntR polypeptide with a selected Wnt polypeptide, in the presence and absence of a test compound,

measuring the effect of the test compound on the extent of binding between said Wnt and said WntR, and

identifying said compound as effective to alter binding of a Wnt polypeptide to a WntR polypeptide if its measured effect on the extent of binding is above a threshold level.

2. The method of claim 1, wherein said threshold is a 2-fold or greater inhibition of binding.

3. The method of claim 1, wherein said threshold is a 2-fold or greater potentiation of binding.

4. The method of claim 1, wherein said Wnt polypeptide is *wingless* (Wg).

5. The method of claim 1, wherein said WntR polypeptide is Dfz2.

6. The method of claim 5, wherein said WntR polypeptide has the amino acid sequence represented as SEQ ID NO:2.

7. The method of claim 1, wherein said test compound is effective to inhibit binding between the Wnt polypeptide and the WntR polypeptide.

8. The method of claim 1, wherein said test compound is effective to displace the Wnt polypeptide from the WntR polypeptide.

9. The method of claim 1, wherein said WntR polypeptide is expressed on the surface of a cell transformed with an expression vector encoding said receptor.

10. The method of claim 9, wherein said cell is a Drosophila Sneider 2 (S2) cell and said expression vector encodes the WntR polypeptide Dfz2.

11. The method of claim 1, wherein said WntR polypeptide is an N-terminal portion of a full-length WntR polypeptide, said portion including the cysteine-rich amino-terminal domain.

12. The method of claim 11, wherein said portion is a first part of a fusion protein.

13. The method of claim 12, wherein said fusion protein further includes a second portion, said second portion containing the constant domain of human IgG.

14. The method of claim 1, further comprising preparing a pharmaceutical preparation of a compound identified as effective to alter binding of a Wnt polypeptide to a WntR polypeptide.

```
Dfz2
Dfz1        MRHNRLKVLI LGLVLLLTSC RADGPLHSAD HGMGGMGMGG HGLDASPAPG   50
Consensus   MWRQILFILP -TLIQGVQRY -DQSPLDASP YYRSGGGL-- --M---ASSG   41
            M....L..L. ..L..... ....PL.... ....G.G... .........G   50

Dfz2
Dfz1        YGVPAIPKDP NLRCEEITIP MCRGIGYNMT SFPNEMNHET QDEAGLEVHQ  100
Consensus   TELDGLPHHN --RCEPITIS ICKNIPYNMT IMPNLIGHTK QEEAGLEVHQ   89
            .....P.... ..RCE.ITI. .C..I.YNMT ..PN...H.. Q.EAGLEVHQ  100
Dfz2        FWPLVEIKCS PDLKFFLCSM YTPICLEDYH KPLPVCRSVC ERARSGCAPI  150
Dfz1        FAPLVKIGCS DDLQLFLCSL YVPVC-TILE RPIPPCRSLC ESAR-VCEKL  137
Consensus   F.PLV.I.CS .DL..FLCS. Y.P.C..... .P.P.CRS.C E.AR..C...  150

Dfz2
Dfz1        MQQYSFEWPE RMACEHLPLH GDPDNLCMEQ PSYTEAGSGG SSGGSGGSGS  200
Consensus   MKTYNFNWPE NLECSKFPVH GGED-LCVAE ------NTTS SASTAATPTR  180
            M..Y.F.WPE ...C...P.H G..D.LC... .......... S.........  200

Dfz2
Dfz1        GSGSGGKRKQ GGSGSGGSGA GGSSGSTSTK PCRGRNSKNC QNPQGEKASG  250
Consensus   SVAKVTTRKH ------QTGV ---------- ---------- ESPH--RNIG  202
            .......RK. ........G. .......... .......... ..P......G  250

Dfz2
Dfz1        KECSCSCRSP LIFLGKEQLL QQQSQMPMMH HPHHWYMNLT VQRIAGVPNC  300
Consensus   FVC------P V------QL- --KTPLGMG- -----Y-ELK VG-GKDLHDC  229
            ..C......P .......QL. .......M.. .....Y..L. V........C  300

Dfz2
Dfz1        GIPCKGPFFS NDEKDFAGLW IALWSGLCFC STLMTLTTFI IDTERFKYPE  350
Consensus   GAPCHAMFFP ERERTVLRYW VGSWAAVCVA SCLFTVLTFL IDSSRFRYPE  279
            G.PC...FF. ..E......W ...W...C.. S.L.T..TF. ID..RF.YPE  350

Dfz2
Dfz1        RPIVFLSACY FMVAVGYLS- ---------R N-FLQNEEIA CDGLL--LRE  387
Consensus   RAIVFLAVCY LVVGCAYVAG LGAGDSVSCR EPFPPPVKLG RLQMMSTITQ  329
            R.IVFL..CY ..V...Y... .........R ..F.......  ..........  400

Dfz2
Dfz1        SSTGPHSCTL VFLLTYFF-G MASSIWWVIL SFTWFLAAGL KWGNEAITKH  436
Consensus   GHRQTTSCTV LFM-ALYFCC MAAFAWWSCL AFAWFLAAGL KWGHEAIENK  378
            ......SCT. .F.....F.. MA...WW..L .F.WFLAAGL KWG.EAI...  450

Dfz2
Dfz1        SQYFHLAAWL IPTVQSVAVL LLSAVDGDPI LGICYVGNLN PDHLKTFVLA  486
Consensus   SHLFHLVAWA VPALQTISVL ALAKVEGDIL SGVCFVGQLD THSLGAFLIL  428
            S..FHL.AW. .P..Q...VL .L..V.GD.. .G.C.VG.L. ...L..F...  500

Dfz2
Dfz1        PLFVYLVIGT TFLMAGFVSL FRIRSVIKQQ GGVGAGVKAD KLEKLMIRIG  536
Consensus   PLCIYLSIGA LFLLAGFISL FRIRTVMKTD G-----KRTD KLERLMLRIG  473
            PL..YL.IG. .FL.AGF.SL FRIR.V.K.. G........D KLE.LM.RIG  550

Dfz2
Dfz1        IFSVLYTVPA TIVIGCYLYE AAYFEDWI-- ------KALA CPCAQVKGPG  578
Consensus   FFSGLFILPA VGLLGCLFYE YYNFDEWMIQ WHRDICKPFS IPCPAARAPG  523
            .FS.L...PA ....GC..YE ..:F..W... ......K... .PC.....PG  600

Dfz2
Dfz1        K---KPLYSV LMLKYFMALA VGITSGVWIW SGKTLESWRR FWRRLLGAPD  625
Consensus   SPEARPIFQI FMVKYLCSML VGVTSSVWLY SSKTMVSWRN FVERLQGKEP  573
            .....P.... .M.KY..... VG.TS.VW.. S.KT..SWR. F..RL.G...  650

Dfz2
Dfz1        RTGANQALIK QRPPIPHPYA GSGMGMPVGS AAGSLLATPY TQAGGASVAS  675
Consensus   RT-------- -R---AQAYV ---------- ---------- ----------  581
            RT........ .R......Y. .......... .......... ..........  700

Dfz2        TSHHHLHHHV LKQPAASHV        694
Dfz1        --------- --------          581
Consensus   .........                   719
```

Fig. 1

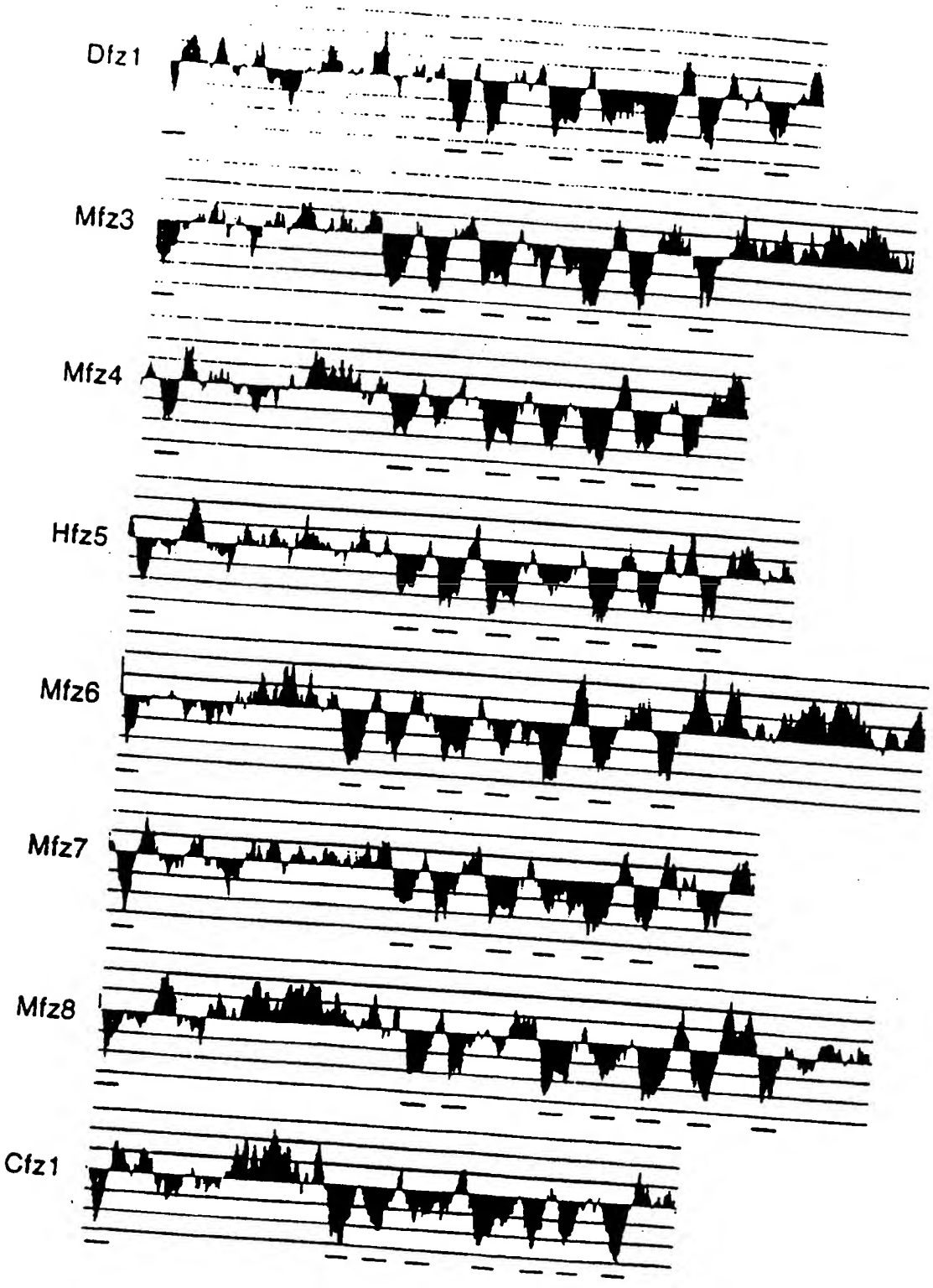

Fig. 2

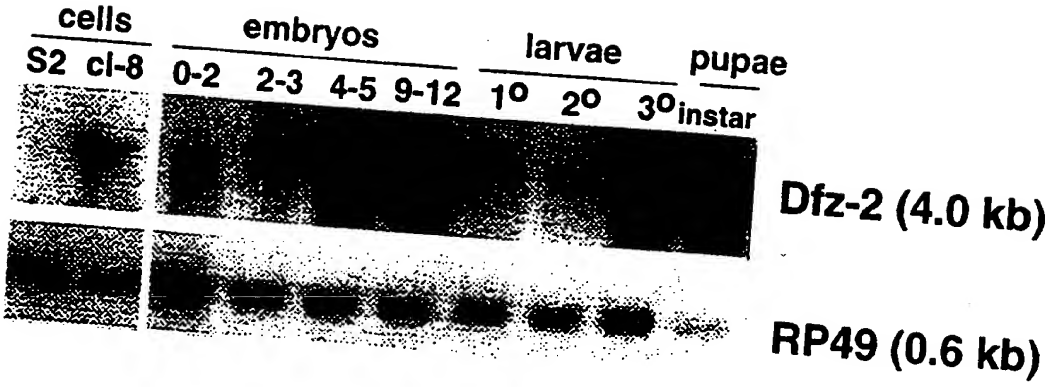
cells
embryos
larvae
pupae
S2
cl-8
0-2
2-3
4-5
9-12
1°
2°
3°
instar
Dfz-2 (4.0 kb)
RP49 (0.6 kb)

Fig. 3

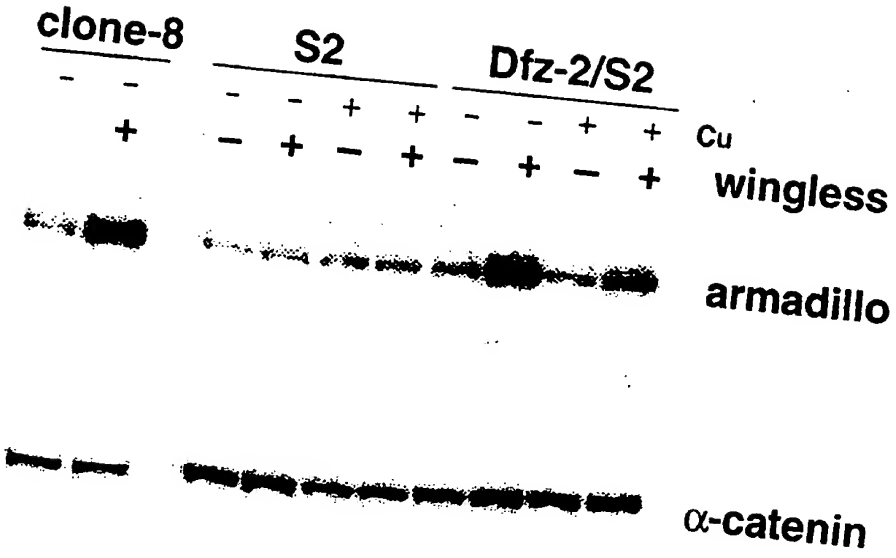

Fig. 4

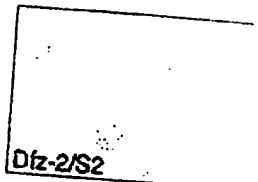

Fig. 5A

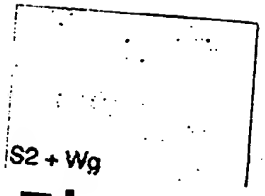

Fig. 5B

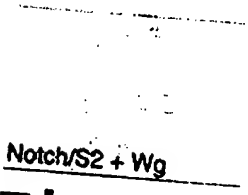

Fig. 5C

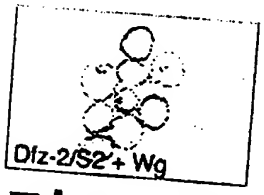

Fig. 5D

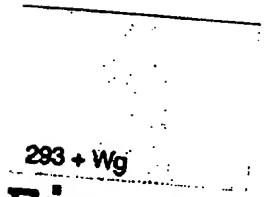

Fig. 5E

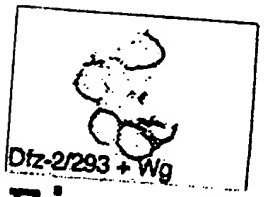

Fig. 5F

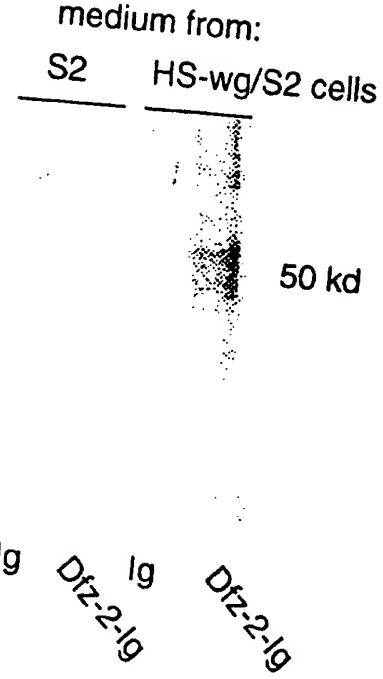

Fig. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/06049

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :G01N 33/566; C12N 15/12; A61K 38/19; C07K 14/705
US CL :435/7.2, 69.1, 69.7; 424/85.1; 530/350, 351
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/7.2, 69.1, 69.7; 424/85.1; 530/350, 351

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN, CAPLUS, MEDLINE, APS, DIALOG, PIR50, A-GENESEQ26, SWISS-PROT34
search terms: Wnt, wingless, Dfz2, receptor, IgG, S2 cells

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 95/17416 A1 (MERCK & CO., INC.) 29 June 1995 (29.06.95), see page 11, lines 14-29 and EXAMPLE 8.	1, 7-9 and 14 ----- 2 and 3
A,P	US 5,585,087 A (LUSTIG et al.) 17 December 1996 (17.12.96).	1-14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
03 JUNE 1997

Date of mailing of the international search report
08 JUL 1997

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
DARYL A. BASHAM
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*